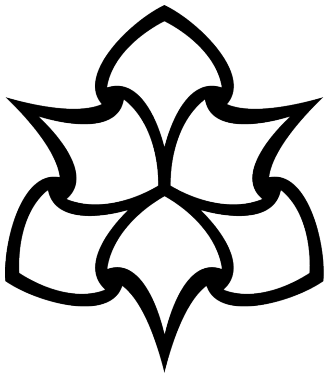

A neuromuscular and kinematic description of human adults with Achondroplasia

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A thesis submitted in partial fulfilment of the requirements of the Manchester Metropolitan University for the degree of Doctor of Philosophy

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To Mum, Dad and Emma.

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Publications

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Chapter 4: Sims, D. T., Onambélé-Pearson, G. L., Burden, A., Payton, C., & Morse, C. I. (2018) 'The oxygen consumption and metabolic cost of walking and running in adults with Achondroplasia.' *Frontiers in Physiology*, *9* (410), pp. 1-8 https://doi.org/10.3389/fphys.2018.00410

Chapter 5: Sims, D. T., Onambélé-Pearson, G. L., Burden, A., Payton, C., & Morse, C. I. (2018) 'Specific force of the vastus lateralis in adults with Achondroplasia.' *Journal of Applied Physiology*, 124, pp. 696-703 https://doi.org/10.1152/japplphysiol.00638.2017

Chapter 6: Sims, D. T., Onambélé-Pearson, G. L., Burden, A., Payton, C., & Morse, C. I. (2018) 'Morphological and mechanical properties of the human patella tendon in adult males with Achondroplasia.' *Frontiers in Physiology*, 9 (867), pp. 1-12 https://www.frontiersin.org/articles/10.3389/fphys.2018.00867/full

Chapter 7: Sims, D. T., Onambélé-Pearson, G. L., Burden, A., Payton, C., & Morse, C. I. (2018) 'A quantitative description of self-selected walking in adults with Achondroplasia using the gait profile score.' *Gait & Posture*, In Press.

Conference proceedings:
Sims, D. T., Onambélé-Pearson, G. L., Burden, A., Payton, C., Jarvis, H. and Morse, C. I. (2017) *An increase in oxygen consumption are associated with changes in gait profile scores in healthy adults but not achrondoplasia (dwarfism).* XXVI Congress of the International Society of Biomechanics, Brisbane Convention & Exhibition Centre Brisbane, Australia

List of Abbreviations

Definition	Abbreviation
Analysis of variance	ANOVA
Abduction at toe off	A9
Anatomical cross-sectional area	ACSA
Anterior cruciate ligament	ACL
Anterior pelvic tilt at initial heel contact	P1
Body fat percentage	BF%
Body mass index	BMI
Body segment parameters	BSPs
Bone mineral content	BMC
Bone mineral density	BMD
Bone mineral density of the lumbar 1-4 vertebrae	BMD_{LUM}
Centre of mass of the body	CoM_B
Centre of mass of the segment	CoM_S
Coefficient of determination	R^2
Correlation coefficient	r
Cross sectional area	CSA
Cross sectional area of the Patella Tendon	CSA_{PT}
Dual X-ray absorptiometry	DEXA
Electromyography	EMG
Eversion at toe off	A8
Fat free mass	FFM
Fibroblast growth factor receptor 3	FGFR3
Flexion at initial heel contact	H1
Flexion at initial heel contact	K1
Flexion at toe off	K3
Force at the patella tendon	F_{PT}
Froude's Number	*Fr*
Gait Deviation Index	GDI
Gait Profile Score	GPS
Gait variable scores	GVSs
Ground reaction force	GRF

Growth hormone deficient	GHD
Heart rate	HR
Internal rotation at heel contact	H8
Internal rotation at toe off	H9
Interquartile range	IQR
Intraclass correlation	ICC
Isometric maximal voluntary contraction	iMVC
Isometric maximal voluntary contraction torque	iMVCτ
Knee extensors	KE
Knee flexors	KF
Lean body mass	LBM
Magnetic resonance imaging	MRI
Maximal heart rate	HR_{max}
Maximal oxygen consumption	$\dot{V}O_{2max}$
Maximal voluntary contraction	MVC
Metabolic cost	C
Metabolic cost of running	C_R
Metabolic cost of running relative to fat free mass	C_{RFFM}
Metabolic cost of running relative to total-body mass	C_{RTBM}
Metabolic cost of walking	C_W
Metabolic cost of walking relative to fat free mass	C_{WFFM}
Metabolic cost of walking relative to total-body mass	C_{WTBM}
Mitogen activated protein kinase	MAPK
Movement analysis profile	MAP
Non-dimensional normalised	NDN
Oxygen consumption	$\dot{V}O_2$
Oxygen consumption relative to fat free mass	$\dot{V}O_{2FFM}$
Oxygen consumption relative to total-body mass	$\dot{V}O_{2TBM}$
Peak abduction at heel contact	A5
Peak abduction during stance phase	H4
Peak abduction during stance phase	A7
Peak abduction during swing phase	H6
Peak adduction during stance phase	H5
Peak adduction during swing phase	H7

Peak anterior pelvic tilt during stance phase	P2
Peak anterior pelvic tilt during swing phase	P3
Peak eversion at heel contact	A4
Peak eversion during stance phase	A6
Peak extension at toe off	H3
Peak extension during stance phase	H2
Peak external rotation during stride	P7
Peak flexion during stance phase	K2
Peak flexion during swing phase	K4
Peak internal rotation during stride	P6
Peak oxygen consumption	$\dot{V}O_{2peak}$
Peak pelvic drop during swing phase	P5
Peak pelvic rise during stance phase	P4
Peak plantarflexion during stance phase	A2
Peak varus angle during stance phase	K6
Peak varus angle during swing phase	K7
Phosphatidylinositol phosphate-3-kinase-serine/threonine kinase and protein kinase B	PI3K-AKT
Phospholipase C *y*	PLCy
Physiological cross-sectional area	PCSA
Plantarflexion at initial heel contact	A1
Plantarflexion at toe off	A3
Posterior cruciate ligament	PCL
Root mean square	RMS
Self-selected walking speed	SSW
Signal transducer and activator of transcription 1	STAT1
Standard deviation	SD
Testing metabolic rate	RMR
Three-Dimensional	3D
Varus angle at toe off	K5
Vastus lateralis	VL
Vertical ground reaction force	vGRF
Volumetric bone mineral density	BMD_{VOL}
Total-body mass	TBM

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Abstract

Skeletal dysplasia is an umbrella term used to describe individuals with an abnormality to the skeleton. There are more than 350 reported skeletal dysplasias with the more common name for the term being 'dwarfism'. The prevalence of skeletal dysplasia is around 1/5000 child births (Bonafe et al., 2015), the most common form of which being Achondroplasia (Krakow and Rimoin, 2010). Individuals with Achondroplasia are termed 'rhizomelic' (i.e. distal appendicular segments are longer than the adjoining proximal segments) and have a disproportionate limb length-to-torso ratio compared to age matched adults of average stature (controls). Average stature of an adult male with Achondroplasia is ~1.30 m while females with Achondroplasia are ~1.25 m (Horton et al., 1977; Hunter et al., 1996). The cause of Achondroplasia is well documented and is attributed to a mutation in the fibroblast growth factor receptor 3 (FGFR3) during foetal growth (Bellus et al., 1995b; Bellus et al., 1995a; Horton and Lunstrum, 2002; Horton, 2006; Horton et al., 2007). Medical and psychological complications associated with Achondroplasia are described, but no data exist on the physiological or biomechanical descriptions of the condition. The aim of this thesis was therefore to quantify the neuromuscular and biomechanical properties of adults with Achondroplasia. Specifically, this would be achieved by measuring: *in vivo* total-body and segment composition; maximal oxygen consumption ($\dot{V}O_{2max}$); submaximal oxygen consumption ($\dot{V}O_2$) and metabolic cost (C) during incremental exercise; neuromuscular and biomechanical properties of muscle and tendon during isometric maximal voluntary contraction (iMVC), and;

lower limb and centre of mass (CoM) kinematics during a range of walking and running speeds, all of which would be compared to controls.

Results showed that the adult group with Achondroplasia had disproportionate legs and arms lengths compared to torso length but were not rhizomelic. Total-body mass, bone mineral content, bone mineral density and fat free mas was lower in the group with Achondroplasia compared to controls. Fat mass however, was higher in the group with Achondroplasia than controls when relative to total-body values; differences in body composition values were lessened somewhat when relative to total-limb values. A lower absolute $\dot{V}O_{2max}$ was observed in the group with Achondroplasia when compared to controls. This difference was removed when presented relative to total-body mass and fat free mass. The group with Achondroplasia had a higher $\dot{V}O_2$ and C than controls at all walking and running speeds, with a persistent higher C being observed when normalised to total-body mass and leg length. The group with Achondroplasia were weaker than controls when presented as absolute values and when accounting for biomechanical and physiological properties (here as specific force). Furthermore, a more compliant patella tendon during iMVC was observed in the group with Achondroplasia compared to controls. Following three-dimensional gait analysis, a number of discrete differences in joint kinematics existed between groups when walking and running, resulting in the group with Achondroplasia being more 'flexed' than controls throughout the walking and running stride. A global analysis of gait kinematics (here as gait profile score) showed that the gait of individuals with Achondroplasia

quantifiably different to controls during walking, with a more similar gait pattern being observed between groups when running.

Body morphology differences between the groups helped normalise the differences in a number of functional measures. However, the persistently higher C in individuals with Achondroplasia during walking and running is likely due to the combination of their lower force development of the knee extensors, their more compliant patella tendon and differences in their gait kinematics compared to controls.

Chapter 1: Literature review

1.1 Introduction to Dwarfism

Skeletal dysplasia is an umbrella term used to describe a range of disorders that affect the developing bone or cartilage in foetal growth and adolescence (Krakow and Rimoin, 2010). There are a reported 372 skeletal dysplasias which are defined by radiographic, biochemical and/or by molecular differences, of which dwarfism is the most common trait (Superti-Furga and Unger, 2007; Bonafe et al., 2015). Dwarfism is primarily characterised by short stature with an individual's body being either disproportionate (i.e. shorter or longer limbs relative to torso length) or proportionate (i.e. limbs and torso are smaller but in proportion to that of an average statured person) (Nehme et al., 1976; Hecht et al., 1987; Hecht et al., 1988; Hunter et al., 1996a; Horton and Lunstrum, 2002; Horton et al., 2007). Achondroplasia is the most prevalent dwarfism within humans, affecting between 1/10,000 and 1/30,000 live births (Horton et al., 2007; Superti-Furga and Unger, 2007; Krakow and Rimoin, 2010).

1.1.1 An overview of Achondroplasia

Achondroplasia was identified as having a genetic link in the 1960's (Langer Jr et al., 1968) but it was not until the 1990's where the locus of mutation was mapped to chromosome 4p16.3 (Rousseau et al., 1994; Shiang et al., 1994; Bellus et al., 1995a). Further work has since identified a mutation to fibroblast growth factor receptor 3 (FGFR3) as the cause of Achondroplasia (Bellus et al., 1995b; Tavormina et al., 1999). FGFR3 is one of four fibroblast growth factors (FGFR1-4) that are found in mammals (Ornitz and Marie, 2002). All have an extracellular ligand-binding domain, a

transmembrane domain and an intracellular domain containing a split tyrosine kinase subdomain. The differing aspects of the receptors are their spatial and temporal distribution, with FGFR3 being expressed later in embryonic development (Delezoide et al., 1998). Similar mutations occur within FGFR1-4, with an amino acid substitution of Gly380Arg in the transmembrane domain being the predominate cause of Achondroplasia. The penetrance of the Gly380Arg mutation is 100% (Horton et al., 2007) while a mutation of Gly375Cys has also been linked to Achondroplasia (Superti-Furga et al., 1995).

In non-chondrocytic cells, such as the colon and bladder, FGFR3 promotes mitosis (Jang et al., 2001), whereas in the growth plates of chondrocytes the effect is converse. Linear bone growth is regulated by FGFR3, acting as an inhibitor to the proliferation and differentiation of growth plate chondrocytes (Deng et al., 1996). The mutated FGFR3 that causes Achondroplasia amplifies the signalling of the cellular pathway, stunting bone growth. As described in Horton (2007), and shown in Figure 1.1, there are four main signalling pathways FGFR3 code for: signal transducer and activator of transcription 1 (STAT1); mitogen activated protein kinase (MAPK); phospholipase C *y* (PLC*y*), and; phosphatidylinositol phosphate-3-kinase-serine/threonine kinase and protein kinase B (PI3K-AKT). STAT1 is described to inhibit chondrocyte proliferation (Sahni et al., 1999), while MAPK adversely affects terminal differentiation, proliferation and post-mitotic matrix synthesis via p38 and extracellular signal-regulated kinase pathways (Murakami et al., 2004). Consequentially, the phenotypes of an individual with Achondroplasia are significant non-linear bone growth and reduced bone length. While the genetic differences to

individuals without Achondroplasia and individuals with other types of skeletal dysplasia are well established, there are few valid reports on the anthropometric variation of skeletal dysplasic conditions and even fewer reports on the functional ability of individuals with Achondroplasia compared to age matched able bodied individuals (hereafter referred to as 'controls').

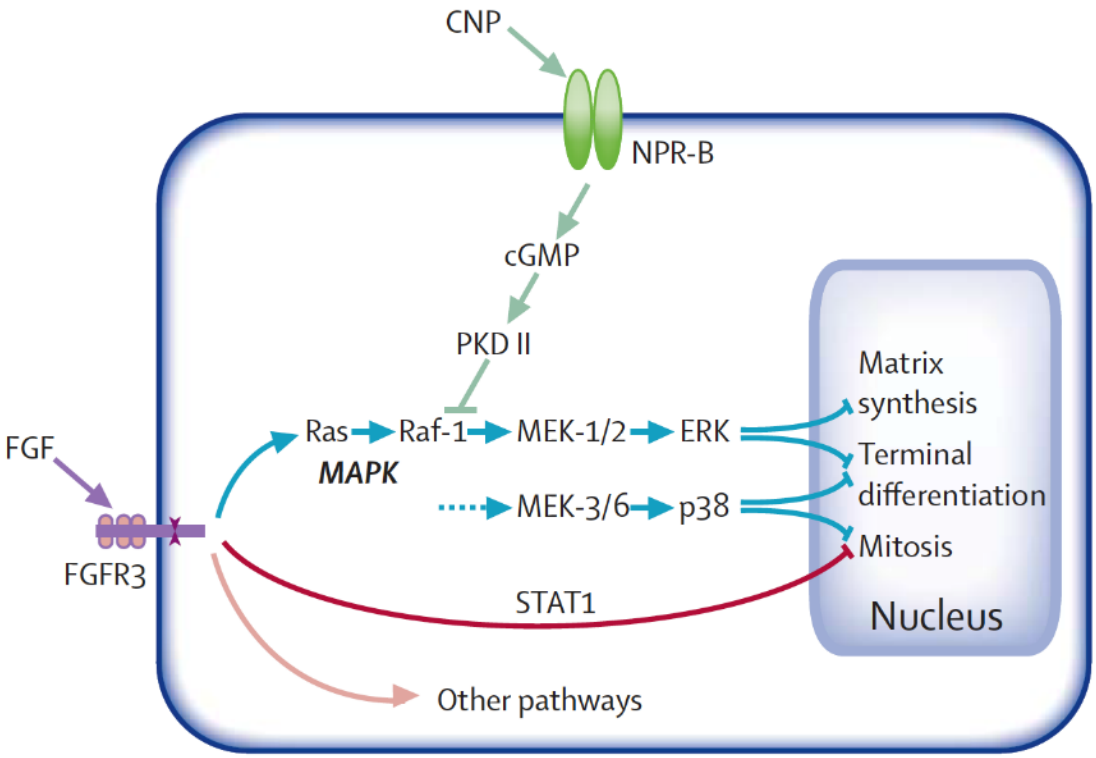

Figure 1.1: Signalling pathways of FGFR3 most relevant to growth plate Chondrocytes. FGFR3 signals propagated through STAT1, MAPK-ERK, MAPK-p38, and other pathways inhibit chondrocyte proliferation, post-mitotic matrix synthesis, and terminal (hypertrophic) differentiation. The CNP-NPR-B pathway inhibits the MAPK pathways. Based on the work by Horton (2006) and taken from Horton (2007).

Individuals with Achondroplasia are classed as having a physical disability (Haga, 2004) with Longmuir and Bar-Or (2000) describing people with disabilities as being less physically active than abled bodied individuals. Longmuir and Bar-Or's (2000) grouping of 'physical impairment' does not incorporate individuals with Achondroplasia, but based on the definitions by Wheeler et al. (2003) and the description of the condition by Haga (2004), individuals with Achondroplasia would be classed in this bracket. The amount of physical activity that individuals with Achondroplasia undertake is unknown. Able-bodied people that are less physically active exhibit increased fat mass and reduced muscle mass compared to more physically active counterparts (Janssen et al., 2002; Rolland et al., 2007). Higher fat mass has been attributed with greater overall body loading however, and therefore greater absolute muscle mass (Tomlinson et al., 2014a). Furthermore, able-bodied individuals who are less physically exhibit a reduction in maximal oxygen consumption ($\dot{V}O_{2max}$) (Laaksonen et al., 2002), maximal voluntary contraction of muscle (MVC) and tendon compliance compared to those that are more physically active (Reeves et al., 2003a; Morse et al., 2007b). Measures such as these can be used as predictors of health status and risk of clinical conditions, such as the metabolic syndrome (Wennberg et al., 2013). Furthermore, reduced strength and lower tendon compliance can negatively impact $\dot{V}O_2$ and metabolic cost (C) during walking and running (Saunders et al., 2004; Fletcher et al., 2010), all of which are unreported in adults with Achondroplasia.

To place an individual on a health continuum, a large reference database that closely matches that of the individual is required to make an informed evaluation. For example, body mass index (BMI) is one such variable that is affected by the morphology of Achondroplasia. BMI incorporates total-body mass (TBM) and stature into its calculation. Although the TBM of an individual with Achondroplasia is lower than controls (Hecht et al., 1988; Hunter et al., 1996a; Hoover-Fong et al., 2007), the difference in stature between individuals with Achondroplasia and controls appears to be greater than the difference in body mass between the groups. Individuals with Achondroplasia are ~25% shorter and ~21% lighter than that of age matched (16-years-old) average statured individuals (Nehme et al., 1976; Hecht et al., 1988; Hoover-Fong et al., 2007). Thus, BMI is skewed and categorises individuals with Achondroplasia as 'obese' on BMI scales derived from able-bodied controls (Owen et al., 1990; Hunter et al., 1996a; Hoover-Fong et al., 2007). Given the disproportionate anthropometric measures of individuals with Achondroplasia compared to controls, appropriate presentations of any anthropometric, physiological, neuromuscular or biomechanical data is required to give an accurate representation of individuals with Achondroplasia relative to controls. For example, in shorter statured groups, fat free mass (FFM) and muscle volume are useful to present relative values of $\dot{V}O_{2max}$ and MVC (Bottinelli et al., 1997; Y. J. Janssen et al., 1999; Goran et al., 2000; Tolfrey et al., 2006; Morse et al., 2008; O'Brien et al., 2010c; O'Brien et al., 2010d; Dencker et al., 2011; Lolli et al., 2017), whereas leg length is a useful scaling factor for gait speed (Holt et al., 1991; Hof, 1996; Steudel-Numbers and Tilkens, 2004; Vaughan and O'Malley, 2005; Steudel-Numbers et al., 2007).

To date, there do not appear to be extensive anthropometric, physiological, neuromuscular or biomechanical data for adults with Achondroplasia. Particularly, accurate assessment of total-body and segmental composition of lean body mass (LBM), bone mineral content and density (BMC and BMD respectively) and body fat percentage (BF%), using methods such as dual X-ray absorptiometry (DEXA), would help enhance the clinical definition of Achondroplasia. In addition, any improvement of the perceived physical limitations associated with the Achondroplasia is centred predominantly around increasing the individual's stature which involves invasive surgical procedures with relatively minor gains in stature (Aldegheri et al., 1988; Cattaneo et al., 1988; Price, 1989; Horton et al., 1992; Nishi et al., 1993; Ganel and Horoszowski, 1996; Shohat et al., 1996; Yasui et al., 1997; Seino et al., 2000; Aldegheri and Dall'Oca, 2001; Vaidya et al., 2006; Tanaka et al., 2010; Park et al., 2015). Such interventions are based entirely on the outcome measure of 'increasing stature' and very few comment on the functional aspects of individuals with Achondroplasia pre- or post-operative measures. Therefore, any functional measures, such as oxygen uptake ($\dot{V}O_2$) during graded exercise, neuromuscular and morphological properties and tendon compliance during MVC or gait analyses pre-operation would undoubtedly aid in a desired outcome of the condition, or even improvement of quality of life, following surgery, if indeed surgery is warranted.

The aim of this review is therefore to discuss and critically analyse the available anthropometric data for populations with Achondroplasia. With a lack of available functional data in populations with Achondroplasia, other shorter stature related populations will be discussed and critically analysed in relation to how stature may

influence physical function. Where possible, data related to populations with Achondroplasia will be reviewed.

1.2 Anthropometry

Anthropometry is a discipline associated with the measurement of physical characteristics of the human body. The primary purpose is to understand the variation that exists between individuals or groups of different anecdotal, physical or medical states (Utkualp and Ercan, 2015). There is a wealth of anthropometric knowledge of the able bodied and average statured human body, yet there are limited empirical anthropometric measures of adults with Achondroplasia. Shorter stature is the overwhelming phenotype for individuals with Achondroplasia which is the manifestation of the mutated of FGFR3 gene (Horton et al., 2007; Superti-Furga and Unger, 2007; Krakow and Rimoin, 2010; Bonafe et al., 2015). For most cohorts, a shorter stature incurs a lower TBM and therefore a lower value to some, or all, derivatives of TBM, such as LBM, BF% and bone mass. Measures of limb lengths in ages 3-18 yrs (Nehme et al., 1976; Horton et al., 1978a; Owen et al., 1990), stature during maturation (Ponseti, 1970; Horton et al., 1978a), mass-to-stature (Hunter et al., 1996a) and mass-to-age (Hoover-Fong et al., 2007) are presented for juvenile and adolescent populations with Achondroplasia. BMD data (Arita et al., 2013; Taşoğlu et al., 2014; Matsushita et al., 2016) and skinfold thickness estimates (Hecht et al., 1988; Owen et al., 1990) also exist for adults with Achondroplasia. The following sections within anthropometry will therefore critically evaluate the available data on the limb lengths of populations with Achondroplasia and in turn discuss how limb

lengths may affect the composition and distribution of their TBM and segmental masses.

1.2.1 Limb length and mass

The average stature of a fully mature male and female with Achondroplasia is ~1.31 and ~1.24 m respectively (LP, 2015). Despite the clear stature differences, Achondroplasia is referred to as a 'rhizolemic' condition, a term which refers to disproportionate appendicular segment lengths (i.e. upper arms and thighs are shorter than forearms and shanks) and therefore a disproportionate limb-to-torso length ratio relative to controls (Ponseti, 1970; Hoover-Fong et al., 2007; Horton et al., 2007; Krakow and Rimoin, 2010). For individuals with Achondroplasia, leg length is of particular importance as this not only defines some of the individual's stature, but also determines gait speed. Despite this, there only appears to be two papers which quantify the length of lower limb segments in individuals with Achondroplasia. Nehme et al. (1976) provided femur and tibia lengths in males and females with Achondroplasia aged between 3 and 18-years-old, while Horton et al. (1978a) measured total-limb length in 403 males and females with Achondroplasia. Both papers confirmed a shorter appendicular limb in the groups with Achondroplasia compared to controls, with Nemhe showing that their groups with Achondroplasia were ~8 standard deviations below controls. Horton showed that the leg lengths of 18-year-olds with Achondroplasia were ~0.30 m shorter than controls (~0.50 m and ~0.80 m, respectively). These data undoubtedly show that lower limb length was the determining factor of shorter stature of individuals with Achondroplasia compared to controls. Nemhe's paper partially confirmed the rhizolemic lengths between thigh

and shank in that tibias are longer than femurs in individuals with Achondroplasia. Horton et al. however, measured total-limb length in individuals with Achondroplasia and did not present comparative data. Despite the overwhelming difference in limb length between individuals with Achondroplasia and controls presented by Nemhe et al. and Horton et al., the quantification of limb length in both papers are dubious as the methodologies are somewhat flawed. These methodological limitations are expanded upon below.

Firstly, Horton et al. (1978b) did not describe the method used to measure limb lengths, only that "measurements of total height, upper and lower segment" were made, and that "most of the measurements were casual and retrospective". Nemhe et al. (1976) on the other hand used x-rays to measure limb length in individuals with Achondroplasia. Anecdotally, the standing position of an individual with Achondroplasia is different to that of a control (Ponseti, 1970). This is likely to affect the perspective error of the measured bones in a single plane, but this was not highlighted on by Nemhe. Secondly, Horton and colleagues included 403 individuals with Achondroplasia that included 189 males between the ages of 2 and 18-years-old. Assuming the participants were equally distributed within this age range, most would have been pre-mid pubertal, and very few classified as post-pubertal or adult. This was similar to Nemhe's study which included only three individuals with Achondroplasia that were between 15 and 18-years-old, thus not providing substantial data for the mature adult with Achondroplasia. Lastly, Nemhe et al. only presented data as standard deviations (SD) and did not report the absolute limb length of any age group nor were any inferential statistics performed on these data.

While segment lengths of individuals with Achondroplasia is well beyond the 1.96 SD needed for a significant difference against control limb length, to confirm the rhizomelic presentation of limb length, statistical analysis would have been useful. Horton et al. (1978b) did provide mean (SD) data for their group with Achondroplasia and controls, but did not provide any group demographics for either, nor were any inferential statistical analyses conducted in this study and therefore omitted any description of age groups.

Whilst there are large differences in leg length and therefore stature between individuals with Achondroplasia and controls, the differences between the groups' TBM is much smaller, with individuals with Achondroplasia being ~1.5 SD lighter than controls (Hoover-Fong et al., 2007). Clinically, the disproportionate stature and mass differences between individuals with Achondroplasia and controls make direct comparisons between groups descriptively important, but functionally irrelevant. For example, the disproportionate mass to stature in individuals with Achondroplasia lead to a BMI calculation that misclassifies the weight category of the individual. Currently, BMI classify a large proportion of the population with Achondroplasia as 'obese' or 'over-weight', but this is based on control observations (Owen et al., 1990; Hunter et al., 1996a; Matsushita et al., 2016). Functional measures in individuals with Achondroplasia, such as $\dot{V}O_2$ during walking, may also be misinterpreted when compared to controls due to the TBM differences between groups. Furthermore the conversion of the same $\dot{V}O_2$ into the C will be affected by the shorter legs of the individuals with Achondroplasia length (Holt et al., 1991; Minetti et al., 1994; Hof, 1996; Minetti et al., 2000; Vaughan and O'Malley, 2005). Presenting functional

measures relative to stature or TBM alone overlooks the fundamental disproportionate mass and stature differences between groups with Achondroplasia and controls. Total, and segmental, body mass are used widely as scaling factors during functional measures in controls, but as discussed above, may not be appropriate to present relative functional measures in individuals with Achondroplasia. To make more valid conclusions of functional measures or to define health statuses in individuals with Achondroplasia, using the constituent compositions of the individual's TBM and segment mass as a scaling factor would be appropriate to help classify health status. The subsequent section outlines other anthropometric factors that are meaningful in clinical and functional comparisons.

1.3 Body composition

Obesity is a growing epidemic in western civilisation and is associated with numerous cardiovascular conditions and physical impairments which affect functional measures such as muscle strength and gait (Browning et al., 2006; Browning et al., 2007; Tomlinson et al., 2014a). Measures of body composition, primarily body fat accumulation, can help predict risk of cardiovascular disease, metabolic syndrome and reduced life expectancy (Després et al., 2006; Després, 2006; Schneider et al., 2006; Brambilla et al., 2013; Freedman et al., 2013). It is generally accepted that populations with Achondroplasia are 'obese' (Hecht et al., 1988; Owen et al., 1990; Hunter et al., 1996a; Hoover-Fong et al., 2007; Horton et al., 2007), but many of the current classifications of obesity in populations with Achondroplasia are based on BMI rather than body composition measures. Given the disproportionate mass-to-

stature in individuals with Achondroplasia compared to controls, and the lack of reliable direct measures of BF% or fat mass distribution in in the population, current classifications of obesity in individuals with Achondroplasia may be inaccurate. The following section will therefore outline the current methods adopted for measuring body composition, specifically BF%, BMC and muscle mass in individuals with Achondroplasia.

1.3.1 Body mass index

A commonly used measure of estimating one's health status in relation to adiposity is BMI (Brambilla et al., 2013; Lo et al., 2016). Calculated as the ratio of TBM to stature^2, BMI is arguably a valid non-invasive estimate of body fat in average statured individuals (Gallagher et al., 1996). Due to the denominator of BMI being stature however, any abnormalities in this value, such as that seen in individuals with Achondroplasia, lead to higher values of BMI. Hunter et al. (1996a) showed that the BMI of 409 children with Achondroplasia were consistently above the 97th percentile range of the general population. More recently, Hoover-Fong et al. (2007) produced a BMI curve including 280 children with Achondroplasia, with results again consistently showing that the BMI of children with Achondroplasia was greater than 50% of controls. However, the BF% of the participants included in Hoover-Fong's paper was not reported.

There is a large amount of BMI data on prepubescent and maturing children with Achondroplasia, but little is available for the adult population. From the available data in adults (~26-years-old) with Achondroplasia, males and females have a BMI

~30 kg·m^{-2} (Owen et al., 1990; Matsushita et al., 2016). A separate case study by Taşoğlu et al. (2014) however, reported a 39-year-old female with Achondroplasia had a BMI of 24.3 kg·m^{-2} (Table 1.1). Given the differences BMI between ages and sexes in control populations, the BMI value for this individual was surprisingly lower than the populations with Achondroplasia included in Owen et al. (1990) and Matsushita et al. (2016). It is pertinent to note though, that the individual with Achondroplasia in Taşoğlu et al. (2014) had undergone leg lengthening. The denominator of the BMI equation was therefore larger for this individual, thus lowering their BMI value. This only emphasises the fact that BMI is an inappropriate measure for populations with Achondroplasia.

The BMI results from above suggest that adults with Achondroplasia are 'overweight' (25 – 29.9 kg·m^{-2}), with some being classed as 'obese' (≥ 30 kg·m^{-2}) (Kelly et al., 2008). With the BMI scale being based on data from averaged statured populations though, the simple method of using anthropometry to describe heath status becomes invalid for the populations with Achondroplasia. Therefore, other methods of attaining total-body or regional body composition in individuals with Achondroplasia, such as BF%, would be more appropriate than the conventional BMI measures.

Table 1.1: Comparisons of anthropometric measures made in individuals with Achondroplasia available in the literature. Data given as mean or mean (SD).

Citation	N	Sex (M/F)	Age (yrs)	Stature (m)	Mass (kg)	BMI ($kg \cdot m^{-2}$)
Hecht et al. (1988)	9	M *	0 - 2	0.749 ‡	11.8 †	21.1 (3.2) †
	13	M *	3 - 4	0.866 ‡	15.8 †	21.0 (4.2) †
	7	M *	5 - 6	0.927 ‡	18.2 †	21.2 (1.8) †
	6	M *	7 - 8	1.021 ‡	21.7 †	20.8 (2.6) †
	12	M *	9 - 10	1.03 ‡	27.1 †	25.4 (8.4) †
	9	M *	11 - 12	1.19 ‡	33.0 †	23.3 (2.1) †
	11	M *	13 - 14	1.32 ‡	40.4 †	23.2 (3.1) †
	16	M *	15 - 16	1.29 ‡	46.8 †	28.0 (7.5) †
	11	M *	17 - 18	1.33 ‡	51.2 †	28.9 (30.1) †
	47	M *	> 19			32.1 (5.0) †
Owen et al. (1990)	15	M *	30 (6)	1.36 (0.05)	57.7 (14.2)	31 (7)
Hunter et al. (1996)				50	4.1 †	16.5 ‡
				60	6.7 †	18.6 ‡
				70	9.6 †	19.5 ‡
				80	12.7 †	19.8 ‡
				90	16.4 †	20.3 ‡
				100	35.9 †	35.9 ‡
				110	28.6 †	23.6 ‡
				120	39.5 †	27.4 ‡
				130	54.4 †	32.2 ‡
				140	61.0 †	31.1 ‡
Hoover-Fong et al. (2007)		M *	16		50.1 †	
Arita et al. (2013)	11	6 M 5 F	40 (8)			NBMD 33.2 (9.4) LBMD 34.3 (5.6
Taşoğlu et al. (2014)	1	F	39	1.25	38	24.3
Matsushita et al. (2016)	18	9 M 9 F	M 17 (7) F 22 (9)			M 24.5 (4.4) F 23.2 (4.8)

M, males; F, females; M *, males and females included in original study, but only males included in this table; †, estimated using ImageJ; ‡, calculated from estimated means; NBMD, normal bone mineral density; LBMD, low bone mineral density

1.3.2 Adiposity

Adiposity is the amount of fat mass a person has in the body and can be presented as a TBM value but is more commonly given as a BF%; throughout this Chapter fat mass is referred to as BF%, unless stated. Numerous methods have been developed to assess *in vivo* BF% in average statured able-bodied populations. Whilst this list is not exhaustive, commonly used techniques include: three-to-nine site skinfold assessments (Durnin and Womersley, 1974; Jackson and Pollock, 1985; J. Wang et al., 1994), bioelectrical impedance (Kyle et al., 2004) and absorptiometry (Levine et al., 2000; Glickman et al., 2004). For individuals with Achondroplasia, absorptiometry may be the only valid measure of BF% from the above list. For example, skinfold assessments were developed in proportionally sized individuals and therefore pose two main issues when used in individuals with Achondroplasia. Firstly, the accuracy in identifying anatomical landmarks in individuals with Achondroplasia is likely to be lower than for controls due to the irregular growth of their endplates. Secondly, the assessment of fat mass using skinfolds is done using known distributions of fat in the control body; distributions of which are currently unavailable in individuals with Achondroplasia. Combining these factors would only increase error when converting sum of skinfolds from individuals with Achondroplasia into BF%. Despite this, skinfold measures have been used to calculate BF% in populations with Achondroplasia (Hecht et al., 1988; Owen et al., 1990).

Although Hecht et al. (1988) were the first to measure skinfold thickness in individuals with Achondroplasia, Owen et al. (1990) were the first to attempt to predict BF% using skinfold measures in adults with Achondroplasia (30 (6) year-olds,

~20% body fat). Owen et al. compared skinfold measures to hydrostatic weighing in their group with Achondroplasia and found the methods to be very close, with hydrostatic weighing giving an average ~21% body fat in the same group. The similarity between these methods are unsurprising since skinfold measures are based on regression equations derived from underwater densitometry measures (Durnin and Womersley, 1974; Jackson and Pollock, 1985). Hunter et al. (1996a) followed suit by measuring the skinfold thickness in the scapula, abdominal and triceps of 409 individuals with Achondroplasia, but did not report on the BF%, instead total skinfold measures were correlated with stature and mass. Unsurprisingly, the total skinfold measures of the individuals with Achondroplasia correlated well with mass but not stature. Owen et al.'s work is useful as it appears to be the only study to report BF% estimates in any adult group with Achondroplasia. However, the large confidence intervals of skinfold methods reported by Jackson and Pollock (1985), whose method was used by Hunter et al. and Owen et al., may somewhat under- or over predict the actual BF% in their respective groups with Achondroplasia. This was partially observed by Owen et al., as BF% in their male group with Achondroplasia varied by 33-88%. With such a large variance in predicted BF% using skinfolds, an absorptiometry estimate, such as that given by DEXA would give a more valid measure of BF% compared to skinfold measures in individuals with Achondroplasia.

Individuals with Achondroplasia are more likely to be classed as 'over-weight' or 'obese' and with their limb and torso lengths being disproportionate compared to controls (Hecht et al., 1988; Owen et al., 1990; Hunter et al., 1996a), the distribution of their BF% is likely to be different throughout their body compared to controls. It

would therefore be useful to provide a breakdown of individual body segment parameters (BSPs) comprising of LBM, BMC, BMD and BF% from groups with Achondroplasia. Such data would help in more accurately defining clinical states for populations with Achondroplasia. For example, android-gynoid ratio of abdomen adiposity is linked to cardiovascular disease risk in controls (Samsell et al., 2014; Okosun et al., 2015), but unknown in individuals with Achondroplasia. Therefore, segmental analysis may be better at indicating, or comparing, health statuses when comparing to controls' data. Furthermore, segmental data could be used to appropriately normalise functional measures of individuals with Achondroplasia to controls. For example, using FFM, calculated by subtracting BMC from LBM, is a useful tool to present $\dot{V}O_2$ when comparing different body morphologies (Goran et al., 2000; Tolfrey et al., 2006; Dencker et al., 2011; Lolli et al., 2017).

DEXA affords the ability to assess total-body and BSPs more accurately than other methods (Durkin and Dowling, 2003; Glickman et al., 2004). DEXA provides additional data on BMC and LBM, which can be used to indicate osteoporotic and sarcopenic states, respectively (Després et al., 1990; Després et al., 2006; Després, 2006; Bianchi, 2007; Bolotin, 2007). To date, DEXA has not been used for the measurement of total-body BF% in adult groups with Achondroplasia, nor to measure BSPs in the same groups. Given that the condition Achondroplasia is defined in part by bone formation, assessment of bone quality using DEXA would also be invaluable for the maintenance of bone health in the condition.

1.3.3 Bone mineral density

An estimate of bone fracture risk, through the measurement of BMD, is based on age and sexed matched comparisons and can be assessed in numerous ways, most commonly using DEXA (Kröger et al., 1995; Genant et al., 1996). Despite the knowledge that the genetic mutation that causes Achondroplasia is associated with impaired growth and development of long bones, there is very limited understanding of how FGFR3 influences measures of 'bone quality', here defined as BMD. It appears that only three studies have measured BMD in individuals with Achondroplasia. Arita et al. (2013) and Matsushita et al. (2016) measured BMD of the lumbar vertebra (L1-4) of 11 and 18 individuals with Achondroplasia respectively; Arita also measured BMD of the mandible in a mixed sexed cohort. Matsushita et al. (2016) identified that the average Z-score of the individuals with Achondroplasia was -1.1 below that of controls whereas Arita et al. (2013) showed that only 6 of the 11 participants with Achondroplasia were of a 'normal' range. Taşoğlu et al. (2014) provided a three-year case report of a 39-year-old female with Achondroplasia and showed that her BMD Z-scores were consistently lower the 3-year period (-1.6 to -1.7). The collective results suggest individuals with Achondroplasia would be considered 'osteopenic' or 'osteoporotic' when compared to controls (T. L. Kelly et al., 2009).

While DEXA is an accurate measure of BMD, this measurement is a ratio between the measured BMC and the viewable area (given as $g \cdot cm^{-2}$). Indeed, it is likely that less BMC would be presented in individuals with Achondroplasia compared to age matched controls due to the genetic mutation affecting bone development. Given that areal BMD ($g \cdot cm^{-2}$) is dependent on bone size and bone content, it is likely that

comparisons to controls will be lower due to the lower bone mass and therefore lower BMC of individuals with Achondroplasia. Volumetric measures of BMD (BMD_{VOL}) however, is a more appropriate scalar of BMC as the depth of bone is taken into consideration (Bianchi, 2007). Particularly for cylindrical shaped bones, such as the lumbar vertebrae, BMD_{VOL} calculations are a more valid method of normalising BMD values between groups of different sizes (Jergas et al., 1995; Kröger et al., 1995; Lang et al., 1997). While vertebral BMD is available in groups with Achondroplasia, BMD_{VOL} observations appear not to have been made. Furthermore, no total-body measure of BMD has been made in any cohort of individuals with Achondroplasia using any technique. Certainly, a normalised method of BMD, such as BMD_{VOL}, would help identify any fracture risk in groups with Achondroplasia compared to controls. A normative total-body BMD data set is required for populations with Achondroplasia though, so that informed comparisons within the group can be made. These data do not currently exist.

1.3.4 Lean body mass

As discussed in the previous section, there is a positive correlation between measurements of stature and mass in populations with Achondroplasia and controls (Ponseti, 1970; Nehme et al., 1976; Hecht et al., 1988; Hunter et al., 1996a; Hoover-Fong et al., 2007). For a control individual, around 70% of their TBM is LBM and consists of muscle, tissue and bone mass (Imboden et al., 2017). LBM is measurable with the use of DEXA, which can also present LBM without BMC, allowing for an estimation of FFM. Roughly 58% of TBM is FFM with muscle mass contributing to

~37% of TBM (Clarys et al., 1984). For this and proceeding sections, muscle mass will be referred to as 'FFM' (i.e. LBM – BMC), while combined LBM and BMC will be referred to as 'LBM'.

An accurate estimation of FFM is useful for both clinical and functional measures. For instance, sarcopenia is defined by FFM, while force production can be correlated with, and presented relative to, FFM (Maughan et al., 1983; Rosenberg, 1989; Narici et al., 1992; Narici and Maffulli, 2010; Stebbings et al., 2014). Given that the trend of absolute LBM is relatively consistent through the ages of 20-85 years in Caucasian controls (Imboden et al., 2017), it could be hypothesised that the trend of LBM would be consistent through the same ages in Caucasian individuals with Achondroplasia . It could also be assumed that absolute LBM would be lower in populations with Achondroplasia given their shorter limb lengths compared to controls. Indeed, this is reported in Owen et al. (1990) with their adult group with Achondroplasia displaying less absolute total-body and LBM mass compared to controls. However, ~84% of the TBM was made up of LBM in the individuals with Achondroplasia, ~14% higher than controls (Imboden et al., 2017). This suggests that individuals with Achondroplasia may have more FFM or BMC than controls as a ratio of TBM. However, the method of hydrostatic weighing does not pertain to individual measures of LBM or BMC. While the ratio FFM to TBM in individuals with Achondroplasia appears high compared to controls, it may be due in part to unexplained variance in the hydrostatic measure of LBM. Comparing the measured TBM (using scales) to the summation of LBM (kg) and fat mass (kg) from Owen's group with Achondroplasia, a mean increase of 3 kg is observed in hydrostatic weighing, equivalent to 4.6% of their

TBM. Therefore, the accuracy of LBM in individuals with Achondroplasia presented by Owen et al. (1990) may not be externally valid for the entire population with Achondroplasia.

For populations with Achondroplasia, any measure of FFM using DEXA would be more valid than hydrostatic weighing and therefore be more useful in scaling some functional measures, such as $\dot{V}O_{2max}$ or strength. For more specific measures of function however, relative values of total-body variables are not accurate for the site-specific measure. For example, strength measures of one muscle group may be under- or over-estimated when relative to TBM. Measures of muscle volume or cross-sectional area (CSA), are more appropriate parameters to present relative values of strength.

Muscle volume and muscle CSA both correlate positively with MVC in controls (Maughan et al., 1983; Bruce et al., 1997) and help normalise strength deficits in smaller statured groups compared to taller groups, such as children and adults (Morse et al., 2008; O'Brien et al., 2010c; O'Brien et al., 2010d) and individuals with growth hormone deficiency (GHD) and controls (Sartorio and Narici, 1994; Sartorio et al., 1995; Bottinelli et al., 1997; Y. J. Janssen et al., 1999). Furthermore, single muscle measures, such as CSA, allow for a more accurate description of clinical states where reductions in lower limb skeletal muscle size contribute to physical impairments, such as sarcopenia (Narici and Maffulli, 2010). Single muscle measures of CSA can be achieved using magnetic resonance imaging (MRI), but can also be obtained using ultrasonography, with the two methods correlating well when

measuring both muscle volume and muscle CSA (Esformes et al., 2002; Reeves et al., 2004c; Morse et al., 2007a). For groups with Achondroplasia, such measures would be useful given their rhizomelic limb lengths and likely muscle length differences compared to controls. To date however, there appears to be no such use of DEXA, MRI or ultrasonography to quantify FFM or muscle volume in individuals with Achondroplasia. Such measures would be useful to more accurately define clinical states or help normalise functional assessments in individuals with Achondroplasia.

1.4 Functional measures

For individuals with Achondroplasia, anthropometric measures are essential in describing the potential clinical implications of the condition. In conditions of short stature, such as Achondroplasia, measures of functional ability are much more relevant for understanding how the condition may impact daily life. In the broadest terms, one of the most frequently adopted clinical measures is 6-minute walk distance; the results of which are explained somewhat by variation of lower limbs strength in clinical groups (Headley et al., 2002; Camarri et al., 2006). For adults with Achondroplasia, the information obtained from the 6-minute walk test is likely compromised not just by strength but by leg length. Indeed, the shorter legs of individuals with Achondroplasia are likely to have relatively reduced muscle mass and in turn reduced force production, but leg length directly influences gait speed and is likely to reduce the distance covered by the individual in the 6-minute walk test (Holt et al., 1991; Hof, 1996; Vaughan and O'Malley, 2005). Therefore, either appropriate tests are required to more appropriately describe functional ability or used to scale

specific parameters of functional measures in individuals with Achondroplasia. The following sections will therefore identify functional measures commonly observed n control populations in three distinct sections: muscle strength; $\dot{V}O_2$, and; gait kinematics.

1.4.1 Neuromuscular impairments

Muscle strength, defined here as the measurement of torque or force during a MVC (isometric, isotonic or isokinetic), represents one of the primary determinants of activities of daily living, walking performance and longevity (Seco et al., 2013; Silva et al., 2014). In many clinical populations, MVC force is reduced in the knee extensors (KE) (Sartorio et al., 1995; Bottinelli et al., 1997; Y. J. Janssen et al., 1999; Reeves et al., 2004b; Reeves et al., 2004a; Meldrum et al., 2007) and plantarflexors (Anker et al., 1997; Morse et al., 2005a; Hussain et al., 2014; Morse et al., 2015). This is consistent in the KE of children with Achondroplasia when compared to controls (Takken et al., 2007), but no data exists in any muscle group for adults with Achondroplasia.

Invariably, individuals of shorter stature generate smaller MVCs than their taller counterparts, while mainly between juvenile and adolescent (Seger and Thorstensson, 2000), adolescent and adult (Morse et al., 2008) and juvenile and adult (Lambertz et al., 2003; Grosset et al., 2005; Grosset et al., 2008). However, when MVCs are normalised to TBM (De Ste Croix et al., 2003; Lambertz et al., 2003) and anatomical CSA (ACSA) of muscle (Kanehisa et al., 1994; Kanehisa et al., 1995;

Lambertz et al., 2003; Morse et al., 2008), these differences are lessened and at times statistically similar. While TBM and muscle size can account for some of the differences in the absolute MVC between cohorts, the determinants of force production include neurological, biomechanical and architectural properties (Maganaris et al., 2001). The following sections will therefore discuss the determinants of force production with emphasis on scaling force production between different statured populations.

1.4.1.1 Determinants of force production

1.4.1.1.1 Muscle size

It is well documented in control populations that muscle size is a key determinant of force production, with larger muscles producing more absolute force (Maughan et al., 1983; Fukunaga et al., 1996; Bruce et al., 1997; Stebbings et al., 2014). In neuromuscular disorders or disease states, such as individuals with Cerebral Palsy (Hussain et al., 2014), disuse (Reeves et al., 2005), immobilisation (Grosset and Onambélé-Pearson 2008; Bostock et al., 2017b; Bostock et al., 2017a), ageing (Narici et al., 2003; Reeves et al., 2004a; Morse et al., 2005a), multiple sclerosis (Onambélé and Degens, 2006) and individuals with Muscle Dystrophy (Morse et al., 2015), the notion is concurrent. It is well established that the primary determinant of MVC force is physiological CSA (PCSA), defined as force per unit area of muscle perpendicular to the muscle fibres (force ÷ fascicle length). MVC torque on the other hand is determined primarily by muscle volume (torque ÷ muscle volume). One would therefore expect individuals with short stature, such as those with Achondroplasia,

to experience reduced force and torque production due to a smaller limb and therefore smaller PCSA and muscle volume, respectively.

Muscle volume, ACSA and PCSA all emphasise a proportional down scaling of muscle size and strength to stature. For example, as an absolute measure, adults with GHD (i.e. proportionally smaller than controls but of the same maturity), have a ~20% lower KE MVC torque than controls (Sartorio and Narici, 1994; Sartorio et al., 1995; Bottinelli et al., 1997; Y. J. Janssen et al., 1999), while children's KE MVC force (O'Brien et al., 2010c; O'Brien et al., 2010b) and ankle plantarflexor MVC force are 54% and 42% less than adults, respectively (Morse et al., 2008). In individuals with GHD, the CSA of the quadriceps (Bottinelli et al., 1997) and quadriceps volume (Y. J. Janssen et al., 1999) have been used to scale KE MVC torque to allow better comparisons to controls (Table 1.2). In children, similar observations are made with PCSA accounting for discrepancies in absolute force production compared to adults (Morse et al., 2008; O'Brien et al., 2010c) (Table 1.2). For individuals with Achondroplasia, any proportional downscaling of torque or force may be skewed due to their disproportionate morphology compared to controls, a factor not reported by Takken et al. (2007) in their group of children with Achondroplasia.

Based on the muscle size and force production of other shorter statured groups, it is likely that adults with Achondroplasia will produce less force from any appendicular muscle group than controls. Any discrepancies in MVC force production from individuals with Achondroplasia will be predominantly determined by muscle mass. However, factors affecting force production, such as neurological, architectural and

biomechanical properties, may compensate for the likely disproportionate muscle mass in individuals with Achondroplasia. These properties of force production are considered in the following sections.

1.4.1.1.2 Neural function

The ability to recruit skeletal muscle of prime movers, here described as activation, positively correlates with MVC torque production. During the same movement, antagonist muscles work to control the moving limb, such as the hamstrings during KE. The magnitude of antagonist recruitment, here described as coactivation, reduces the impact of the MVC torque production by the prime movers (Macaluso et al., 2002) and therefore is an important factor to consider when measuring MVC torque or force. A general trend of increased coactivation and decreased activation is observed in individuals that have a neurologic impairment, people that are less physically active and the elderly compared to controls; these differences in activation and coactivation profiles contribute almost entirely for the lower MVC torque compared to controls (Amiridis et al., 1996; Häkkinen et al., 1998; Morse et al., 2005b; Hussain et al., 2014). In individuals with Achondroplasia, the level of agonist activation or coactivation during MVC is unreported but may highlight possible weaknesses of the population.

1.4.1.1.3 Agonist activation (activation)

In healthy groups there is evidence that, depending on the muscle group, muscle activation is between 82-90% during MVC, increasing more so in physically active groups (Babault et al., 2001). Neither stature, nor muscle mass (Tomlinson et al., 2014b), appear to influence activation in adults, with individuals with GHD having similar activation to controls (Sartorio and Narici, 1994; Johannsson et al., 1997). In individuals with neurological impairment however, such as those with Cerebral Palsy, almost all the strength deficit is accounted for by lack of activation compared to controls; this is observed in a group of physically active individuals though (Hussain et al., 2014). In able-bodied populations, physical activity appears to be a major contributor to activation (Amiridis et al., 1996). Coupling the fact that individuals with Achondroplasia are ambulatory and do not appear to have a neurological impairment, there is no reason for their agonist activation to be lower than controls. Therefore, any difference in MVC force between individuals with Achondroplasia and controls is expected to be accounted for by muscle size or coactivation. However, there are no data at present that quantify activation or coactivation levels of any muscle group in individuals with Achondroplasia.

1.4.1.1.4 Antagonist activation (coactivation)

Increased coactivation is usually observed in the presence of compromised muscle strength, joint pain or laxity in clinical groups such as individuals with Cerebral Palsy (Damiano et al., 2000; Elder et al., 2003; Barber et al., 2011; Barber et al., 2012), the elderly (Häkkinen et al., 1998; Macaluso et al., 2002; Reeves et al., 2004a; Morse et

al., 2005b; Morse et al., 2007b) and individuals that have suffered a stroke (Hsiao and Newham, 2001). Coactivation is important in stabilising joints during high torque movements but can also restrict movement of the joint. For example, increased coactivation of the hamstrings is associated with decreased gait speed (Schmitz et al., 2009; Peterson and Martin, 2010) and stair descent time (Larsen et al., 2008) in the elderly compared to younger populations. During KE, the anterior cruciate ligament (ACL) is a structure that aids in knee stability and is put under strain as the tibia is moved anteriorly. Increased coactivation of the hamstrings deters this anterior shift of the tibia allowing for a more stable joint and reduced risk of ACL injury during high KE torque movements (Fairbank et al., 1984). Recent data on the knees of individuals with Achondroplasia shows a reduced congruency and differences in structures compared to controls, such increased ACL-Blumensaat line and posterior cruciate ligament angles (Akyol et al., 2015). Therefore, the coactivation of the knee flexors in individuals with Achondroplasia during KE may be higher than controls to help protect the morphologically different knee joint from injury.

The ability of individuals with Achondroplasia to activate their muscles is most likely similar to controls, but the difference between the groups' knee structure may incur a higher coactivation of hamstrings during high torque KE movements in the individuals with Achondroplasia. This in turn would reduce net torque production of the KE group. Furthermore, a higher coactivation of muscles not only lowers net joint moments in clinical groups, but is also positively associated with a higher $\dot{V}O_2$ during walking (Peterson and Martin, 2010). Therefore, for individuals with Achondroplasia,

any measurement of force or torque production would most likely need to incorporate a description of coactivation profiles to ensure the agonist contribution is represented fully.

1.4.1.1.5 Specific force

Due to muscle force production being multifaceted, normalising force or torque to morphological features only, may be misleading (Maganaris et al., 2001). Recently, calculations have been employed to account for the morphological (muscle size), architectural (pennation and length of fascicles), neurological (activation and coactivation), and biomechanical properties (moment arm) of force production of muscles (Narici et al., 1992; Fukunaga et al., 1996; Maganaris et al., 2001; Morse et al., 2007b; Erskine et al., 2009; O'Brien et al., 2010c; Stebbings et al., 2014). Measured as force per unit area of muscle ($N \cdot cm^{-2}$), specific force normalises to the fascicle level and affords the comparison between pathological and healthy populations. *In vivo* measures of specific force in children (Morse et al., 2008; O'Brien et al., 2010c), individuals with Cerebral Palsy (Hussain et al., 2017), the elderly (Reeves et al., 2004a; Morse et al., 2005a) and healthy populations (Kawakami et al., 1994; Kawakami et al., 1995; Fukunaga et al., 1996; Erskine et al., 2009; Erskine et al., 2011; Stebbings et al., 2014) show comparable results to one another (Table 1.2). *In vitro* measures of specific force in animal tissue (Geiger et al., 2000; Urbanchek et al., 2001; Greising et al., 2013) also show a range of values similar to human tissue (Table 1.2). To the author's knowledge, no measure of specific force production has been made in any muscle group for individuals with Achondroplasia. Therefore it

remains unknown as to whether any difference in MVC force between individuals with Achondroplasia and controls can be accounted for by the composite elements used in the calculation of specific force, such as those observed in other shorter stature group comparisons (Sartorio and Narici, 1994; Sartorio et al., 1995; Bottinelli et al., 1997; Johannsson et al., 1997; Y. J. Janssen et al., 1999; Morse et al., 2008; O'Brien et al., 2010b; O'Brien et al., 2010c). Any differences in specific force between individuals with Achondroplasia and controls would highlight possible differences in biomechanical or physiological mechanisms between the groups, such as myofilament differences (Stebbings et al., 2014), or differences in tendon compliance (Reeves, 2006).

Table 1.2: Comparisons of normalised torque and force production in lower and upper appendicular limbs made in the available literature. Data given as mean or mean (SD).

Reference	Clinical Description	Age (SD) yrs and (N)	Stature (SD) m	Muscle Group	Specific Muscle	Contraction Type	Torque (N·m) or Force (N)	Normalising Method	Absolute Measure (F_T, T, IK)	Specific Force (units of $N \cdot cm^{-2}$ unless stated)
Narici et al. (1992)	Healthy	34 (5) (6)	1.74 (0.04)	QF	VL	Isometric	Force	PCSA	F_T 1412 (532) N	23.7 (1.3)
					VI				F_T 1997 (187) N	24.1 (1.6)
					VM				F_T 1913 (827) N	27.9 (2.0)
					RF				F_T 1601 (306) N	24.3 (2.2)
Sartorio et al. (1994)	GHD ‡	30 (4) (8)	1.46 (0.10)	QF	All QF measured	Isometric	Force *	CSA_{M+B}	F_T 464 (33) N	3.7 (0.5) †
	Healthy (controls)	32 (5) (8)	1.74 (0.04)					Body Mass		10.3 (2.1) $N \cdot kg^{-1}$
	GHD ‡							CSA_{M+B}	F_T 742 (49) N	3.9 (0.5) †
	Healthy (controls)							Body Mass		10.1 (1.9) $N \cdot kg^{-1}$
Sartorio et al. (1995)	GHD ‡	30 (4) (8)	1.46 (0.10)	QF	All QF measured	Isometric	Force *	CSA_{M+B}	F_T 484 (101) N	3.7 (0.5)
	Healthy (controls)	32 (5) (8)	1.74 (0.04)						F_T 747 (136) N	3.8 (0.5)
Fukunage et al. (1996)	Healthy	34 (9) (8)	1.77 (0.06)	DF	TA and EDL measured	Isometric (ankle at 90°)	Force	PCSA (TA only)	F_T 3623 (136) N	10.8 (0.2)
				PF	All PF group measured	Isometric (ankle at 120°)	Force	PCSA (Whole PF group)	F_T 832 (19) N	24.5 (1.1)
Bottinelli et al. (1997)	GHD	30 (2) (5)	1.51 (0.04)	QF		Isometric	Force *	ACSA (muslce and bone)	T 487 (39) N·m	3.29 (0.30) †
	Healthy (controls)	29 (5) (13)	1.76 (0.05)			Isometric	Force *	ACSA (muslce and bone)	T 674 (48) N·m	3.71 (0.18) †
Janssen et al. (1999)	GHD ‡	49 (2) (38)	1.76 (0.03)	QF		Isometric	Force *	Muscle Volume of QF; sum of 12 MRI scan below trochanter minor, 10 mm spacing, from 20 GHD	T 154 (SEM 8) N·m	0.22 (0.01) $N \cdot cm^{-3}$
	Healthy (controls)	Age matched to GHD	Height matched to GHD			Isometric	Force *	Muscle Volume of QF; sum of 12 MRI scan below trochanter minor, 10 mm spacing, from 8 controls	T 185 (SEM 10) N·m	0.22 (0.01) $N \cdot cm^{-3}$
	GHD ‡	As above	As above			Isokinetic	Torque		IK 125 (7) N·m	0.18 (0.00) $Nm \cdot cm^{-3}$
	Healthy (controls)	As above	As above			Isokinetic	Torque		IK 139 (8) N·m	0.17 (0.00) $Nm \cdot cm^{-3}$

Maganaris et al. (2001)	Healthy	28 (4) (6)	1.75 (0.08)	DF	TA	Isometric	Force	PCSA	F_T 458 (36) N	15.5 (1.3)
				PF	Sol				F_T 1783 (118) N	15.0 (1.2)
Reeves et al. (2004)	Rat model (old)	74 (4) (9)	1.63 (0.09)	QF	VL	Isometric	Force	PCSA		27.0 (6.3)
	Healthy (old control)	67 (2) (9)	1.68 (0.12)	QF	VL					23.6 (6.1)
Morse et al. (2005)	Healthy (old)	74 (4) (19)	1.73 (0.04)	PF	Gas L	Isometric	Force	PCSA	F_T 258 (49) N	9.2 (1.9)
	Healthy (controls)	25 (4) (12)	1.76 (0.08)						F_T 416 (56) N	13.1 (2.0)
Morse et al. (2008)	Healthy (Children)	11 (0) (11)	1.45 (0.03)	PF	Gas L	Isometric	Force	PCSA	F_T 243 (52) N	15.9 (2.7)
	Healthy (controls)	25 (4) (12)	1.76 (0.08)						F_T 416 (56) N	13.1 (2.0)
Eskine et al. (2009)	Healthy	21 (3) (27)	1.77 (0.06)	QF	All QF measured	Isometric	Force	PCSA (L_f of VL used only)	F_T 6335 (957) N	30.3 (4.9)
O'Brien et at. (2010)	Healthy (only male children data included for this table)	9 (1) (10)	1.38 (0.09)	QF	All QF measured	Isometric	Force	PCSA (sum of individual QF muscles)	F_T 3699 (853) N	54.1 (14.2)
	Healthy (only male control data included for this table)	28 (4) (10)	1.80 (0.08)						F_T 8037 (1636) N	55.0 (11.0)
Erskine et al. (2011)	Healthy ‡	20 (3) (42)	1.78 (0.06)	QF	All QF measured	Isometric	Force	PCSA (Lf of VL used only)	F_T 5809 (243) N	25.9 (5.3)
Stebbings et al. (2014)	Healthy	21 (3) (73)	1.78 (0.07)	QF	VL	Isometric	Force	PCSA	F_T 6430 (1113) N	23.8 (3.5)

QF, Quadriceps Femoris; θ, pennation angle (°); L_f, fascicle length; VL, vastus lateralis; PF, plantarflexors; Gas L, gastrocnemius Lateralis; PCSA, physiological cross sectional area; GHD, growth hormone defficient; MRI, magnetic resonance imaging; ACSA, anatomical cross sectional area; CSA_{MHB}, cross sectional area of muscle and bone; TA, tibialis anterior; VI, vastus intermedius; VM, vastus medialis; RF, rectus femoris; Sol, Soleus; EDL, extensor digitorum longus; Ext, extensors; Flex, flexors; T Bra, triceps brachii; Bic, bicep brachii; Bra, brachialis; Brd, brachoradialis; * Reports force but does not account for moment arm of joint; ‡ pre intervention; F_T, measure force at the tendon; IK, isokinetic; T, torque; † data presented graphically and estimated using Image J (National Instituite for health, version 1.50i) for this table.

1.4.1.1.6 Tendon properties

The ability to effectively transfer force to the bone relies on the compliance of its tendon. Here tendon compliance is defined as Young's Modulus, which is calculated as the stress through the tendon (tendon force ÷ tendon CSA, given as $N \cdot cm^{-2}$) divided by the strain it experiences (change in tendon length ÷ resting tendon length, given as %). Where a stiffer tendon exists, the rate of force transfer to the bone is high and the converse is observed for a more compliant tendon (Maganaris and Paul, 2000a; Maganaris, 2002; Onambélé et al., 2006; Pearson and Onambélé 2006; Reeves, 2006; Onambélé et al., 2007). Furthermore, the elastic properties of tendon have been linked to postural balance and joint stability (Onambélé et al., 2006; Onambélé et al., 2008).

Joint laxity is clinically observed in infants with Achondroplasia (Bober et al., 2008) and is referred to on numerous occasions in collagen affected skeletal dysplasias and child populations with Achondroplasia (Sillence et al., 1979; Inan et al., 2006; Horton et al., 2007; Venkatesh et al., 2009; Krakow and Rimoin, 2010). However, implications of joint laxity in adults with Achondroplasia are rarely commented on. In elderly control populations, who have a reduced level of physical activity, both a lower force production and tendon compliance are observed (Reeves et al., 2003a; Morse et al., 2005b; Reeves et al., 2005; Onambélé et al., 2006; Onambélé et al., 2008; K. E. Burgess et al., 2009b; Grosset et al., 2014). Male and female pre-pubertal children show a lower tendon compliance compared to sex matched adults (O'Brien et al., 2010b). By accounting for the stress and strain of the tendon during contraction, an estimate of the mechanical properties can be described. Despite this, elderly and

child tendons are more compliant suggesting intrinsic differences to controls, such as fibril density.

Hormonal differences are also associated with lower tendon compliance. For example where higher levels of oestrogen are associated with lower tendon compliance, such as between obese and lean groups and, between sexes (Kubo et al., 2003; Zazulak et al., 2006; B. F. Miller et al., 2007; Onambélé et al., 2007; Hansen et al., 2009; Burgess et al., 2010; Taş et al., 2017). In addition, it is known that visceral fat accumulation (as would occur in obesity) causes dysregulation of adipocyte functions. This in turn leads to a number of cardio-metabolic diseases including hypertension; hypertension itself is associated with elevated levels of relaxin (Papadopoulos et al., 2014). Studies link elevated relaxin levels with higher laxity of ligaments (Dehghan, 2014) and lower tendon compliance (Pearson et al., 2011). For individuals with Achondroplasia, the anecdotal evidence of joint laxity in infants (Bober et al., 2008), indications of obesity in population (Hecht et al., 1988; Horton et al., 1978a; Owen et al., 1990; Hoover-Fong et al., 2007) and probable lower physical activity (Longmuir and Bar-Or, 2000) would indicate a likely lower tendon compliance in the population.

The aforementioned suggestions of tendon compliance in individuals with Achondroplasia are speculative as to date, there are no empirical observations of the tendon made in the population during rest or contraction. If these theories are accurate, their expected lower tendon compliance compared to controls is not only likely to affect force production at the joint, but also the mechanical energy storage

and transfer which in turn affects energy expenditure and $\dot{V}O_2$ during walking and running (Arampatzis et al., 2006; Fletcher et al., 2010). The following sections will therefore discuss the available literature surrounding cardiovascular research in shorter statured individuals.

1.4.2 Cardiorespiratory impairments in shorter statured individuals

Defined as the amount of oxygen utilised in the body, $\dot{V}O_2$ gives an estimate of the energy demands of individuals during steady state exercise (Jones and Carter, 2000). Subtraction of the resting metabolic rate (RMR) from the measured steady state $\dot{V}O_2$ gives net $\dot{V}O_2$ for a given workload, referred to hereafter as '$\dot{V}O_2$'. Presenting $\dot{V}O_2$ per unit distance, rather than a rate, gives exercise C (Morgan et al., 1989; Saunders et al., 2004). While $\dot{V}O_2$ and C are a useful clinical measure to compare groups at submaximal exercise intensities, maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$) is an accurate indicator of cardiovascular health and mortality (Kodama et al., 2009). In juvenile (Rowland et al., 1987; Rowland and Green, 1988b; Rowland, 1993) and clinical groups, such as individuals with renal disease (Moore et al., 1993), a plateau in $\dot{V}O_2$ (which defines $\dot{V}O_{2max}$ (Jones and Carter, 2000)) is rarely observed. This is due to participant fatigue rather than volitional exhaustion, as such the maximal $\dot{V}O_2$ value recorded for these populations is termed $\dot{V}O_{2peak}$. For adults with Achondroplasia though, there is no data that would suggest that $\dot{V}O_{2max}$ could not be attained, particularly as children with Achondroplasia can attain $\dot{V}O_{2max}$ (Takken et al., 2007).

With submaximal $\dot{V}O_2$ and $\dot{V}O_{2max}$ correlating positively with TBM, $\dot{V}O_2$ and $\dot{V}O_{2max}$ can, and has been, presented relative to TBM (and derivatives of TBM, such as FFM) in groups of different morphology (Goran et al., 2000; Weibel and Hoppeler, 2005; Tolfrey et al., 2006; Dencker et al., 2011; Lolli et al., 2017). Leg length on the other hand, influences $\dot{V}O_2$ more so than it does $\dot{V}O_{2max}$. The attainment of $\dot{V}O_{2max}$ occurs at an intensity of exercise that is individualised based on physiological parameters, such as lactate threshold (Pereira and Freedson, 1997), running economy (Jones, 2006) or maximal heart rate extrapolation from graded running (Grant et al., 1995). Submaximal $\dot{V}O_2$ and C however, are measured at set exercise intensities. For example, walking is a common modality and intensity of exercise to assess $\dot{V}O_2$, but leg length determines gait speed (Holt et al., 1991). Therefore, were shorter individuals to walk at the same set speed as taller individuals, a higher C would likely be observed due to a higher stride frequency (Minetti et al., 2000). Incorporating leg length when measuring $\dot{V}O_2$ or C is therefore important to allow comparative values between different statured groups. An example of a geometric scaler is Froude's number (*Fr*), which is a dimensionless speed value that incorporates leg length and helps scale $\dot{V}O_2$ between intra- and inter-specie comparisons (Vaughan and O'Malley, 2005). Incorporation of such geometric (*Fr*) and morphological (TBM or FFM) parameters when measuring C values during walking and running helps rationalise any suggested physiological or biomechanical differences between the observed groups.

For individuals with Achondroplasia, measurements of $\dot{V}O_{2max}$ and C during walking and/or running would allude to cardiovascular health and energy demands of the

population, respectively. Using the conventional methods of presenting $\dot{V}O_2$ or C for groups with Achondroplasia however, may lead to erroneous comparisons to controls. This section of the review will therefore discuss the available literature surrounding the measurement of $\dot{V}O_2$ and $\dot{V}O_{2max}$ in shorter statured individuals and comment on the appropriate morphological and geometric presentations of $\dot{V}O_{2max}$, $\dot{V}O_2$ and C for these groups.

1.4.2.1 Maximal oxygen consumption

Absolute and relative (i.e. presented relative to TBM or FFM) values of $\dot{V}O_{2max}$ are lower in people of shorter stature, but these differences are somewhat lessened when relative to morphological measures. Conventionally, TBM is used to present $\dot{V}O_2$ and $\dot{V}O_{2max}$ as it is a more convenient to measure than FFM. Relative values of $\dot{V}O_{2max}$ using TBM appears to be a useful scaling parameter when utilised within groups of similarly proportioned individuals. There are instances though where differences in absolute $\dot{V}O_{2max}$ between groups of different arthrometry and geometry are not completely removed when relative to TBM. For example, the absolute measure of $\dot{V}O_{2max}$ in African Pygmies is ~49% less than Caucasian controls; this difference is lessened to ~27% when $\dot{V}O_{2max}$ is relative to TBM (Ferretti et al., 1991). The use of FFM to scale $\dot{V}O_2$ or $\dot{V}O_{2max}$ may therefore be argued as more valid than TBM due therefore being stronger relationships between FFM and $\dot{V}O_{2max}$ than TBM and $\dot{V}O_{2max}$ (Batterham et al., 1999). In addition, FFM contributes to ~90% of the measured $\dot{V}O_2$ during intense exercise (Tolfrey et al., 2006).

Both Dencker et al. (2011) and Goran et al. (2000) show that $\dot{V}O_{2max}$ is ~28% lower in obese compared to lean populations, but < 8% lower when relative to FFM in the same populations. Tolfrey et al. (2006) showed that absolute $\dot{V}O_{2max}$ in children was ~50% lower than adults when presented relative to TBM, but was ~3% lower when presented to FFM (this particular study FFM was muscle volume). When FFM is used to present relative values of $\dot{V}O_{2max}$ in individuals with GHD, differences to controls are also supressed (Cuneo et al., 1991; Whitehead et al., 1992; Nass et al., 1995). The lower absolute $\dot{V}O_{2max}$ observed in these shorter statured groups compared to taller counterparts would suggest that adults with Achondroplasia would present a lower absolute $\dot{V}O_{2max}$ than controls. This though, is yet to be measured empirically.

Certainly, FFM appears to appropriately normalise $\dot{V}O_{2max}$ in groups that differ in stature and morphology. Given the reduced stature and probable increased adiposity of individuals with Achondroplasia compared to controls, the use $\dot{V}O_{2max}$ relative to TBM likely becomes moot. Based on the observations described in the previous section and in earlier sections on the morphology of individuals with Achondroplasia, FFM would be a more appropriate variable to present relative values of $\dot{V}O_{2max}$ in individuals with Achondroplasia compared to control values. To date however, there appears to be no attempt to measure $\dot{V}O_{2max}$ in adults with Achondroplasia, and therefore no attempt of scaling such measures.

1.4.2.2 Submaximal oxygen consumption and metabolic cost

A positive curvilinear relationship exists for $\dot{V}O_2$ when presented against incremental walking and running speeds (Rowland and Green, 1988a; Minetti et al., 1994; Schepens et al., 2004; van den Hecke et al., 2007). Whereas, for the same graded intensity that exhibits a positive trend of $\dot{V}O_2$ described above, a U-shaped curve and a negative curvilinear trend in C exists for walking and running respectively (Ferretti et al., 1991; Minetti et al., 1994; McCann and Adams, 2002a). This relationship of $\dot{V}O_2$ is similar in all statures, although proportionally smaller statured people appear to have a higher absolute $\dot{V}O_2$ at set ambulatory speeds (Ferretti et al., 1991; Minetti et al., 1994; Rogers et al., 1995; Minetti et al., 2000; DeJaeger et al., 2001; McCann and Adams, 2002b; Morgan et al., 2002; Schepens et al., 2004; P. A Kramer and Sarton-Miller, 2008; Weyand et al., 2010). This is a consistent finding when observing C. For example, children have ~20% lower walking C relative to TBM compared to adults at matched speeds (McCann and Adams, 2002b). A similar observation is made in groups of children of differing ages with younger, and therefore shorter statured children appearing to have a higher C than older children at set walking intensities (Morgan et al., 2002). This is also apparent in adults with child onset GHD and adult African pygmies who have a higher C than controls and Caucasian controls, respectively, at matched walking speeds (Ferretti et al., 1991; Minetti et al., 1994; Minetti et al., 2002). These results are not surprising given that stride frequency at a set speed is dependent on leg length (Hof, 1996). Therefore, the leg length normalisers when presenting $\dot{V}O_2$ or C values is justified.

As mentioned in section 1.4.2, *Fr* provides a dimensionless speed value by incorporating leg length. Given as velocity^2 ($m \cdot s^{-1}$) $\div \sqrt{\text{leg length (m)} \cdot 9.81\ (m \cdot s^{-2})}$, *Fr* values for humans are ~0.22 at self-selected walking speeds (Hof, 1996; Steudel-Numbers and Tilkens, 2004; Vaughan and O'Malley, 2005; Steudel-Numbers and Weaver, 2006; Steudel-Numbers et al., 2007). The relationship between $\dot{V}O_2$ and *Fr* appears strong for comparisons of inter- and intra-species (Vaughan and O'Malley, 2005). There is certainly a positive linear relationship between *Fr* and $\dot{V}O_2$ in children (DeJaeger et al., 2001), African pygmies (Ferretti et al., 1991; Minetti et al., 1994) and adults with child onset GHD (Minetti et al., 2002) during walking. These relationships match those of adult and control populations, respectively, suggesting the cardiovascular response of shorter statured groups is similar to taller controls at a similar dimensionless speed. Interestingly, the same respective U-shaped and negative curvilinear trends of walking and running C presented against speed is apparent when presented against *Fr*. However, where $\dot{V}O_2$ is similar between groups when incorporating *Fr*, C of shorter statured groups is higher than controls. This suggests biomechanical and physiological differences between groups of different stature (Ferretti et al., 1991; Minetti et al., 1994; Minetti et al., 2002; Schepens et al., 2004; P. A Kramer and Sarton-Miller, 2008; Fletcher et al., 2010). The subtle differences in leg lengths between participants however, means that available $\dot{V}O_2$ and C data presented against *Fr* in shorter statured groups have only been presented descriptively.

For groups with Achondroplasia, the disproportionate lower limb length would likely lead to an overestimation of $\dot{V}O_2$ and C values compared to controls. The correct

scaling of $\dot{V}O_2$ is important when comparing C in individuals with Achondroplasia compared to controls. Differences in $\dot{V}O_{2max}$ and $\dot{V}O_2$ between differing statured groups when walking and running appear adequately scaled when using FFM and *Fr*, but they have not been implemented in groups that are both disproportionate in morphology and stature, such as individuals with Achondroplasia. Once scaled appropriately, any persistent differences in $\dot{V}O_2$ or C between individuals with Achondroplasia and controls would allude to differences in either cardiovascular mechanisms or biomechanical properties between the groups. Certainly, the measurement of the musculotendinous properties described above (section 1.4.1 and its sub sections) may explain some of the potential difference in C between groups. A more likely explanation for the potential differences in C would be the movement of the groups' body's centre of mass (CoM_B) during gait. The measurement and analysis of CoM_B is often conducted by kinematic methods which is the final functional measure reviewed in this literature review.

1.4.3 Gait and kinematics

Kinematics involves the use of two- or three-dimensional motion analysis to predict the movement patterns of individual segments of whole bodies during activity (Cappozzo et al., 2005; Chiari et al., 2005; Della Croce et al., 2005; Leardini et al., 2005). Although walking is one of the most commonly reported measures of physical impairment in clinical populations, timed walking tasks such as the 6-minute walk test do not determine what aspect of gait may be impaired. Kinematic analysis of walking can determine joint angles and joint velocities, allowing the inference of any

differences the individual may have when compared to age matched controls (Cappozzo et al., 2005; McGinley et al., 2009). Gait is predominantly a linear movement and combines coordination and synchronisation of the musculotendon units for an individual to ambulate effectively. While the muscle and tendon work in synchrony to propel the body, to some extent it is the dimensions of the skeleton that determine key gait events, for example the length of the leg determines stride length (Hof, 1996; Steudel-Numbers and Tilkens, 2004; Steudel-Numbers and Weaver, 2006). For otherwise healthy ambulatory individuals, gait at a self-selected walking speed (SSW) appears coordinated and efficient, whereas in groups where pathology exits, anecdotal and empirical kinematic differences are observed (Inan et al., 2006; Egginton et al., 2006; van der Meulen et al., 2008; Baker et al., 2009; Kark et al., 2012; Schweizer et al., 2014). Differences in gait kinematics of many pathological cohorts are explained by neurological impairment, muscle weakness, amputation or deformity (Baker et al., 2009; Kark et al., 2012; Schweizer et al., 2014), but little empirical data exist that extensively describe the gait of populations with Achondroplasia.

There appear to be only four published documents describing gait kinematics of individuals with Achondroplasia, three of which are abstracts. Three studies are conducted in child cohorts, one with an age range between 3-17 years-old (Inan et al., 2006) another with a large age variance, mean 17-years-old SD 16-years, (Egginton et al., 2006) and the last being a case study of a 4-year-old pre- and post-tibial osteotomy surgery (Rethlefsen and Tolo, 1998). The fourth study observed the gait in adults with Achondroplasia following limb lengthening surgery (van der

Meulen et al., 2008). Descriptively, the results of the four studies suggest individuals with Achondroplasia have a shorter stride length, increased pelvic rotation and anterior pelvic tilt throughout the stride compared to controls. Individuals with Achondroplasia also appear to have reduced hip extension and a varus position of the knee during the stance phase, and increased dorsiflexion during the entire stride compared to controls. However, there may be a disparity in comparisons to the adults with Achondroplasia who have not undergone leg lengthening surgery.

There is a plethora of data surrounding short statured gait, but this is predominantly in children of different ages with and without pathologies (Cavagna et al., 1983; Alexander, 1984; Subramanian et al., 1998; DeJaeger et al., 2001; Bell et al., 2002; Rodda et al., 2004; Schepens et al., 2004; van den Hecke et al., 2007; Schwartz et al., 2008). Therefore, any comparisons to populations with Achondroplasia are not comparable due to the leg geometry and joint morphology differences between groups with Achondroplasia and controls (Ponseti, 1970; Nehme et al., 1976; Akyol et al., 2015). Furthermore, the gait related data collected in the three available studies of individuals with Achondroplasia are not sufficient to systematically describe the gait of adults with Achondroplasia (Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008), nor is there any mention of gait quality or indication of potential gait aetiology of populations with Achondroplasia from the available data.

As described above, gait is predominantly a linear movement but encompasses multiple joints moving in all three planes. To understand gait aetiology of a

population, in depth analyses are needed. Differences in single or multiple joints are likely to affect the translation of the CoM_B or be compensated by other joints or limbs during the same phase of gait. The following sections will discuss the use of kinematic data in relation to the calculation of 'gait quality', CoM_B calculations and the relevance of accurate *in vivo* measures of body mass, discussed earlier in this review, to these calculations.

1.4.3.1 Gait profiling

With the large number of kinematic variables that are collected during gait, quantifying whether a person, or population, is different to another is difficult. Recently, methods have been developed to quantify gait kinematics over one stride by encompassing numerous kinematic parameters (Schwartz et al., 2008; Baker et al., 2009). The Gait Profile Score (GPS, Baker et al. (2009)) and the Gait Deviation Index (GDI, Schwartz et al. (2008)) are calculated from 15 kinematic variables and produce a single value which provides a global measure of gait quality. Both the GPS and GDI are derived from the root mean square difference of gait kinematics from a sample population. The two methods therefore present a very strong correlation with one another ($r = 0.995$, (Baker et al., 2009).

The GPS also shows good correlations with clinical assessments ($R^2 = 0.96$ against Gillette functional assessment questionnaire and $R^2 = 0.99$ against gross motor function classification system, Baker et al., (2012)) and high face validity against clinician ratings (Spearman's $r = 0.84$, (Beynon et al., 2010)). Furthermore, GPS is the

sum of the movement analysis profile (MAP) which provides gait variance scores (GVSs) for each of the 15 kinematic variables that make up GPS, GDI only presents one value. Each GVS allows for inter- and intra-joint and plane comparisons which is useful to determine which are the predominant joints affecting gait quality and therefore aid in gait rehabilitation or gait improvement interventions. Lastly, GPS and MAP is derived from angular measurements, giving units of degrees (°). Therefore, GPS can be presented as absolute or relative differences between groups and allows more powerful statistical analyses to be conducted than GDI.

The GPS has been used in adults and children with Cerebral Palsy (Baker et al., 2009; Beynon et al., 2010; Baker et al., 2012), individuals with Down's Syndrome (Galli et al., 2015), patients with varying pathologies, including joint laxity (Schweizer et al., 2014), a modified version in lower limb amputees (Kark et al., 2012) and arm spasticity in individuals who have suffered a stroke (Johansson et al., 2014). The GPS consistently shows that people with pathologies have a different gait compared to age matched controls with individuals GVSs helping to highlight which of the 15 kinematic variables is most different (Table 1.3). Based on previous reports of gait in individuals with Achondroplasia, and with subtle differences in joint morphology between individuals with Achondroplasia, it is likely that their gait is different to controls. Notably, any measure of 'gait quality' cannot be made without first measuring the kinematic profile of the cohort in question. To date, gait kinematics have not been measured in a group of adults with Achondroplasia without limb lengthening and so no data exists that suggests how much their gait differs to controls, if indeed it is different at all.

Table 1.3: Comparisons of gait profile scores made in clinical populations available in the literature. Data given as median (IQR).

Reference	Clinical Description	Age (SD) yrs	N	GPS score (°) median (IQR)
Baker et al. (2009)	271 = CP	12 (3)	407	9.7 (4.9)
	88 = GOC			
	48 = NC			
	TD Children	11 (3)	38	5.2 (1.9)
Beynon et al. (2010)	37 = CP	Range 4-18	60	Summed GPS for all groups:
	11 = GOC			
	10 = NC			15.5 (5.4) †
	2 = Other			
Baker et al. (2012)	271 = CP	12 (3)	407	FAQ 6, 14.3 (4.7)
	88 = GOC			FAQ 7, 11.4 (4.8)
	48 = NC			FAQ 8, 10.9 (4.6)
				FAQ 9, 9.0 (3.5)
				FAQ 10, 7.6 (3.3)
	TD Children	11 (3)	38	5.3 (1.4)
Kark et al. (2012)	TT Prosthetic	62 (13)	11	6.7 (2.3)
	TF Prosthetic	63 (12)	8	10.6 (1.8)
	TT Intact		11	6.5 (2.0)
	TF Intact		8	8.8 (4.0)
	Able-bodied	61 (8)	28	NR
Schweizer et al. (2013)	Obi	16 (9)	176	
	NflaUni	22 (16)	12	
	NflaBi	19 (13)	83	
	NspUni	17 (10)	176	
	NspBi	16 (8)	119	
	NSpBiNTC	19 (10)	57	
	Healthy individuals	25 (12)	102	
Gali et al. (2015)	Down Syndrome	25 (5)	24	11.2 (5.4)
	Healthy individuals	27 (9)	15	4.2 (1.6)

TD, typically developed; FAQ, groups categorised based on functional assessment questionnaire (low FAQ = more impaired), CP, Cerebral Palsy; GOC, general orthopaedic conditions; NC, neurological conditions; NR, not reported; † data presented graphically and estimated using Image J (National Institute for health, version 1.50i) for this table; OBi, spinal disorders; NflaBi, poliomyelitis; NflaBi, spina bifida and ligamentous laxity; NspUni, hemiparesis of various aetiologies; NspBi, bilateral diplegia with trunk control; NspBiNTC, neurological spasticity without trunk control; TT, transtibial amputee; TF, transfemoral amputee.

1.4.3.2 Centre of mass movement

While joint angles can help describe contraction patterns during gait, joint positions allow for the calculation of mass centre positions of individual segments (CoM_S) which in turn can estimate the CoM_B position and translation patterns (Aleshinsky, 1986b; Aleshinsky, 1986a). The changing of joint angles in healthy individuals, alters the translations of the CoM_B (Ortega and Farley, 2005). These findings are also observed in clinical populations, for example individuals with Cerebral Palsy have a greater vertical (van den Hecke et al., 2007; Zollinger et al., 2016) and medio-lateral (Zollinger et al., 2016) CoM_B translation compared to controls. Greater medio-lateral translation of the CoM_B is also observed in the obese and lower limb amputee groups compared to normal weight and abled-bodied groups (Browning et al., 2009; Weinert-Aplin et al., 2017). As observed in clinical groups mentioned here, there is a positive association between increased mechanical work (i.e. movement of the CoM_B) and C (DeJaeger et al., 2001; Mian et al., 2006; Teunissen et al., 2007; Grenier et al., 2012). This association is likely due to the changes in CoM_B translation compared to controls which is likely a result of changes in joint kinematics.

Ultimately the leg length determines the majority of vertical translation of the CoM_B, which translates as an inverted pendulum like motion (Cavagna et al., 1976; Cavagna and Kaneko, 1977; Cavagna et al., 1977). With leg length being shorter in individuals with Achondroplasia compared to controls (Nehme et al., 1976), the absolute height of the CoM_B would be lower in individuals with Achondroplasia compared to controls. In addition, it is likely that the pattern of vertical CoM_B movement would be similar to controls (i.e. local minima and maxima). However, based on the medio-

lateral movements of the CoM_B observed in obese individuals (Browning et al., 2006), the greater adiposity measured in groups with Achondroplasia would suggest a greater medio-lateral movement compared to controls (Hecht et al., 1988; Owen et al., 1990; Hoover-Fong et al., 2007). However, both vertical and medio-lateral movements of the CoM_B are unreported in any group of individuals with Achondroplasia. To determine the position of CoM_B, not only are the coordinates of key joint centres needed, but also segmental masses are required. The following section will address methods of attaining BSPs and their application to populations with Achondroplasia.

1.4.3.3 Body segment parameters (BSPs)

Descriptions of BSPs have been made in males and females (Durkin and Dowling, 2003; Chambers et al., 2010), elderly (Dempster, 1955; Clauser et al., 1969), different morphologies (Damavandi et al., 2009), during pregnancy (R. K. Jensen et al., 1996), children (R. K. Jensen, 1986; R. K. Jensen, 1989) and different ethnic groups (Cheng et al., 2000). BSPs have been determined using anthropometric (Hanavan Jr, 1964; Zatsiorsky and Seluyanov, 1985; De Leva, 1996), cadaveric (Dempster, 1955; Clauser et al., 1969), radiation (Zatsiorsky, 1983; Levine et al., 2000; Durkin et al., 2002) force plate (Pataky et al., 2003; Damavandi et al., 2009) and kinematic techniques (Hatze, 1980), but none have been reported for individuals with Achondroplasia. As discussed in section 1.2, and its subsections, the morphology and geometry of individuals with Achondroplasia is different to controls. A likely misinterpretation of the CoM_B prediction of individuals with Achondroplasia would be made using the

conventional methods of inertial properties of segments, such as Dempster's (1955) adult data or Jenson's (1986; 1989) juvenile data. For individuals with Achondroplasia, *in vivo* measures of BSPs (as described in section 1.3) would be appropriate and useful to help estimate the CoM_B.

1.5 Aims/Objectives

Functional physiological and biomechanical measures have been discussed in depth for many cohorts, but very little data has been provided in the populations with Achondroplasia, with even less being observed in adults with Achondroplasia. Therefore, the overriding aim for this thesis is to present physiological and biomechanical data related to functional tasks in an adult population with Achondroplasia. More specifically, the objectives of the current thesis were therefore to:

1) Report limb lengths and body composition of adults with Achondroplasia and compare to controls;
2) Measure the maximal aerobic capacity ($\dot{V}O_{2max}$) of adults with Achondroplasia and compare to controls;
3) Measure the submaximal oxygen consumption ($\dot{V}O_2$) and metabolic cost (C) of adults with Achondroplasia adults during habitual (SSW) and standardised gait speeds and compare to controls;
4) Describe architectural properties of the muscle-tendon complex during maximal voluntary contraction in adults with Achondroplasia and compare to controls;

5) Provide a lower limb and CoM_B kinematic analysis of gait at habitual (SSW) and standardised speeds in adults with Achondroplasia and compare to controls.

1.5.1 Overview and hypotheses of proceeding chapters

Chapter 2 will describe the *in vivo* morphological and limb length of 15 body segments in a group of adults with Achondroplasia by using absorptiometry and three-dimensional motion analysis. It is hypothesised that:

1) The adults with Achondroplasia will have shorter legs and arms than age matched controls, while head, torso and pelvis will be similar in length between groups;
2) The distribution of muscle and fat mass will differ between groups and segments;
3) The adults with Achondroplasia will exhibit less bone mass (BMC) and lower BMD than controls;
4) No differences in BMD will be observed when scaling BMC to volumetric measures.

Chapter 3 will present the $\dot{V}O_{2max}$ of adults with Achondroplasia and controls. Presentation of absolute $\dot{V}O_{2max}$ will be made relative to TBM and FFM obtained from Chapter 2. The hypotheses are that:

1) The adults with Achondroplasia will have a lower absolute $\dot{V}O_{2max}$ compared to controls;

2) The difference in $\dot{V}O_{2max}$ between groups will decline when $\dot{V}O_{2max}$ is presented relative to TBM and FFM.

Chapter 4 will present the $\dot{V}O_2$ profile of adults with Achondroplasia during habitual gait speed (SSW) and 12 standardised speeds that range from slow walking to fast running on a treadmill. $\dot{V}O_2$ will be presented and converted to C values. For both parameters, morphological (TBM and FFM) and non-dimensional normalisers (i.e. *Fr*) will be used to normalise data. It is hypothesised that:

1) The adults with Achondroplasia will have the same absolute $\dot{V}O_2$ values at each absolute speed and a lower absolute $\dot{V}O_2$ at SSW compared to controls;
2) When relative to TBM and FFM, the adults with Achondroplasia will have a higher $\dot{V}O_2$ at every gait speed compared to controls;
3) Walking and running C will be higher in the adults with Achondroplasia at every gait speed compared to controls;
4) The inclusion of *Fr* into both $\dot{V}O_2$ and C values will help scale the absolute differences between groups.

Chapter 5 will measure the *in vivo* properties of the vastus lateralis during maximal voluntary contraction using an isokinetic dynamometer in adults with Achondroplasia and controls. Specifically, the size, activation profile and architectural properties of the muscle will be measured using ultrasonography. The moment arm of the knee will be obtained using DEXA and be incorporated into the calculation of specific force to account for any differences between groups. It is hypothesised that:

1) The adults with Achondroplasia will have a lower knee extensor torque and force compared to controls;
2) Both knee extensor torque and force will be normalised between groups when incorporating morphological measures of the vastus lateralis;
3) There will be no difference in specific force between groups.

Chapter 6 will encompass data from Chapter 6 to determine the compliance and structure of the patella tendon during maximal voluntary contraction in adults with Achondroplasia and controls. It is hypothesised that:

1) The adults with Achondroplasia will have a shorter and thinner patella tendon than controls;
2) Stiffness and Young's Modulus will be lower in the patella tendon of the adults with Achondroplasia compared to controls.

Chapter 7 will comprise of a three-dimensional kinematic analysis of the same walking and running speeds set in Chapter 4 in adults with Achondroplasia and controls. Data from Chapter 2 will be used to predict the position of the CoM_B during a complete stride. Lastly, the kinematic data will be used to provide a clinical analysis of gait using the global gait score, GPS. It is hypothesised that:

1) Discrete gait kinematics will be different between groups;
2) The adults with Achondroplasia will have a greater GPS score than controls for all speeds;
3) The adults with Achondroplasia will have greater relative CoM_B movement in the vertical and medio-lateral planes compared to controls.

Finally, Chapter 8 will retrospectively view the results of the preceding Chapters. This chapter will draw together the preceding Chapters' findings in an attempt to explain any persistent differences from the preceding Chapters that are not explained by relative presentations of functional data. Lastly, this Chapter will discuss the clinical implications of the collected data from all Chapters and consider prospects for future research within populations with Achondroplasia.

Chapter 2: *In vivo* anthropometric measures of adults with Achondroplasia

2.1 Abstract

Current data suggest that the bone mineral content (BMC) and bone mineral density (BMD) of individuals with Achondroplasia are below age matched individuals of average stature (controls). However, due to the disproportionate limb-to-torso length of individuals with Achondroplasia compared to age matched average stature individuals (controls), the likely statistically lower BMC and BMD may be removed when presented appropriately. There also appears to be a lack of total-body and relative (i.e. ratios of total-body mass) body composition data available for populations with Achondroplasia. The aim of this study was to measure and compare total-body and segmental body composition in adults with Achondroplasia (N = 10, 22 ±3 yrs) and controls (N = 17, 22 ±2 yrs). Dual energy X-ray absorptiometry (DEXA) and three-dimensional modelling was used to respectively measure the *in vivo* masses (BMD, BMC, fat free mass (FFM) and body fat mass) and lengths of the total-body and 15 segments. All variables were presented as an absolute value and then each segment was presented relative to the total-body and total-limb values, respectively. BMC of lumbar vertebrae (L1-4) was also measured and presented as a volumetric BMD (BMD_{VOL}). As an absolute measure, adults with Achondroplasia had shorter limbs, but the same length torso as controls. Absolute measured of BMC, BMD and FFM were lower in the adults with Achondroplasia compared to controls, whereas body fat mass was higher. There was no difference in group's limb lengths when presented to the total-limb length, but the adults with Achondroplasia had a longer foot. When presented relative to total-body and respective total-limb values, BMD was the same between groups but the adults with Achondroplasia had lower

relative BMC values. There was no difference in lumbar BMD_{VOL} between groups. The adults with Achondroplasia have disproportionate limb lengths relative to the torso compared to controls, but the dimensions of the limbs are the same between groups when relative to the total-limb. The adults with Achondroplasia could be classed as 'osteopenic', which would not be apparent when appropriately presented. Further work is needed to create a reliable and referable database for the body composition of populations with Achondroplasia to be compared.

Key words: Achondroplasia; Body Composition; Bone Mineral Content; Bone Mineral Density; Volumetric Bone Mineral Density

2.2 Introduction

Achondroplasia is the most common genetic form of dwarfism and is classically characterised by disproportionate limb-to-torso length and shorter stature (< 1.47 m) compared to controls. Achondroplasia is brought about by a fibroblast mutation resulting in impaired linear long-bone growth (Nehme et al., 1976; Horton et al., 1978b; Horton, 2006; Horton et al., 2007; Baujat et al., 2008; Krakow and Rimoin, 2010). Despite the well-established description of the condition, physiological and biomechanical measurements are poorly represented in the literature. For example, the 'disproportionate' nature of the condition has received little attention beyond case reports with little quantitative confirmation of limb length or body composition (here defined as bone mineral content (BMC), bone mineral density (BMD), fat mass and fat free mass (FFM)) given other than in heterogeneous groups with Achondroplasia.

The 'rhizomelic' limb length (i.e. longer distal segments compared to the adjoining proximal segment) is a common term used to describe the limb lengths of individuals with Achondroplasia (Haga, 2004; Horton et al., 2007; Baujat et al., 2008; Bober et al., 2008; Krakow and Rimoin, 2010; Arita et al., 2013; Matsushita et al., 2016), but there are no robust statistical analyses to confirm this. Furthermore, limb segments have not been presented relative to the limb to quantify their possible proportional shortening. Attempts have been made to describe BMC, BMD, FFM and fat mass in children with Achondroplasia during maturation (Hecht et al., 1988), male and female populations with Achondroplasia of differing ages (Owen et al., 1990; Arita et al., 2013; Matsushita et al., 2016) and case reports (Taşoğlu et al., 2014). However,

the participant inclusion criteria and *in vivo* body mass evaluation methods used in these studies are not robust enough to allow appropriate comparisons to commonly used reference data (hereafter referred to as 'controls') or within differing populations of Achondroplasia (i.e. age and sex).

Considering Achondroplasia is a genetic condition that influences the development of the long-bones, an accurate description of BMC and BMD is essential, but is underreported. Clinically, BMC and BMD are used to describe bone density, quality and strength and, define osteoporosis; a systemic skeletal disease which is characterised by reduced bone tissue (Kanis et al., 1994; Tabensky et al., 1996). There are empirical data from cohorts with Achondroplasia that suggest BMC and BMD in the femur (Su et al., 2010; Taşoğlu et al., 2014), spine (Arita et al., 2013) and mandible (Arita et al., 2013; Matsushita et al., 2016) are lower than controls when presented as Z-scores. Therefore, individuals with Achondroplasia could be at a greater risk of bone fractures. Bone health (here as BMD) is assessed using either Z or T scores but using such methods to assess bone health is difficult in populations with Achondroplasia. There is likely a disproportionate total-body BMC and BMD of individuals with Achondroplasia compared to controls, manifested by disproportional limb-to-torso between groups. Therefore, the commonly used Z and T scores would likely under estimate the bone health of individuals with Achondroplasia were they utilised.

Studies observing BMC and BMD in groups with Achondroplasia have predominantly used DEXA as the mode of data collection (Arita et al., 2013; Taşoğlu et al., 2014;

Matsushita et al., 2016). All BMD measurements using DEXA are given as a ratio to area view. Individuals with Achondroplasia have irregular shaped long bones compared to controls (Ponseti, 1970; Nehme et al., 1976) which may alter the interpretation of bone density, and therefore definition of bone health, when compared to controls. Furthermore, individuals with Achondroplasia are likely to have less bone mass (BMC) and a smaller area view of the bone, due to their shorter bones. Therefore, the calculation of total-body BMD in individuals with Achondroplasia is likely to be inaccurate when compared to controls. The calculation of volumetric BMD (BMD_{VOL}) may be more appropriate to compare the BMD of individuals with Achondroplasia controls as this method considers a greater amount of the observed bone. For example, in groups of shorter stature, BMD was lower but BMD_{VOL} were similar to control groups (Lu et al., 1996; García-Hoyos et al., 2017). It would therefore be useful to describe the BMD_{VOL}, such as the lumbar vertebra, in individuals with Achondroplasia to give a more accurate representation of BMD and quality compared to controls.

Furthermore, it would be expected that individuals with Achondroplasia would have less appendicular and total-body FFM than controls due to their shorter limbs. Conversely though, it appears individuals with Achondroplasia have a higher amount of body fat percentage than controls when assessed with skinfold callipers (Hecht et al., 1988; Owen et al., 1990) and water densitometry (Owen et al., 1990). Regional measures of body fat and FFM in individuals with Achondroplasia would be more useful to the clinician however as regional fat mass of the abdomen is highly correlated with metabolic syndrome and decreased life expectancy (Després et al.,

1990; Després et al., 2006; Freedman et al., 2013). There appears to be, however, no conclusive total-body or segmental body composition measures made in any population with Achondroplasia.

The aims of the current study therefore were to measure segment lengths and to collect *in vivo* total-body and segmental parameters of individuals with Achondroplasia.

The objectives of this study were to:

1) measure 15 individual segment lengths using 3-dimensional movement analysis techniques in adults with Achondroplasia and compare to age matched controls;
2) assess the *in vivo* BMC, BMD, body fat mass and FFM distributions of the total-body and 15 segments using DEXA in adults with Achondroplasia and compare to age matched controls;
3) present segment lengths, BMC, BMD, FFM and body fat to appropriate anatomical measures between groups;
4) use the total-body and relative data to outline any health markers that may exist in adults with Achondroplasia.

2.3 Method

After written informed consent, 10 adult males medically confirmed as exhibiting Achondroplasia and 17 age and sex matched controls agreed to partake in this study (anthropometric descriptions of each group are given in Table 2.1).

Table 2.1: Anthropometric data for the groups of males with Achondroplasia and controls, values displayed as mean (SD).

	Achondroplasia (N = 10)		Control (N = 17)
Stature (m) †	1.38 (0.05)	*	1.79 (0.08)
Age (yrs)	22 (3)		22 (2)
Total-body mass (kg)	61.9 (8.7)	*	76.5 (10.6)
Body Mass Index ($kg \cdot m^{-2}$)	32.4 (3)	*	24.1 (4.5)
BMC (kg)	2.1 (0.3)	*	3.1 (0.5)
BMD ($g \cdot cm^{-2}$)	1.17 (0.10)	*	1.37 (0.11)
FFM (kg)	41.3 (5.3)	*	55.6 (7.6)
Body Fat (kg)	18.3 (3.9)		16.8 (5.0)
Body Fat (%)	29.3 (2.9)	*	22.4 (5.3)
BMC_{LUM} (g)	62.5 (13.8)	*	90.4 (15.3)
BMD_{VOL} ($g \cdot cm^{-3}$) †	0.290 (0.051)		0.279 (0.044)

BMC, Bone Mineral Content; BMD, Bone Mineral Density; FFM, Fat Free Mass; BMC_{LUM}, Bone Mineral Content of the Lumbar Vertebrae (L1-4); † Mann Whitney-U t-test. * $P \leq 0.001$.

The inclusion criteria for all participants were males between the ages of 18-35-years-old, were self-reported as physically active (> 2 hours of structured exercise per week) and were free from injury or aliments that would hinder exercise performance. For the group with Achondroplasia, inclusion criteria also required them to be non-leg lengthened. Based on these criteria, the group with Achondroplasia consisted of ~13% of the registered U.K. population (Burton, 2018). From the literature search conducted for Chapter 1 this group of adult males with Achondroplasia represents the most homogenous skeletal dysplasic group available in the literature (i.e. accounting for age, sex, condition, pre-leg lengthened and activity levels).

Ethical approval *for this thesis* was attained from the local committee (Manchester Metropolitan University, see Appendix 1) and conformed to the latest revision of the Declaration of Helsinki. Each participant attended one testing session at the laboratories of Manchester Metropolitan University where total-body anthropometric measurements were carried out.

2.3.1 Stature and mass

The stretch stature method was used to measure stature of all participants (Tanner, 1962) using a fixed stadiometer with a tolerance ±1 mm (Stadiometer, Harpenden with Veeder-Root high speed counter, Holtain Ltd, Crymych, Wales). Participants were the weighed using electronic scales (SECA 813, CA 91710 Chino, USA) while wearing minimal, light clothing and were barefooted.

2.3.2 Body composition

After fasting for ~8 hrs, a DEXA scanner (Hologic Discovery, Vertec Scientific Ltd, UK) was used to measure total-body mass (kg), BMC (kg), BMD ($g \cdot cm^{-2}$), FFM (kg) and body fat (kg) of the total-body. Participants wore a loose-fitting cotton gown and lay supine in a predefined, anatomical position that ensured enough space was between each arm and the torso, and between each leg. The feet were positioned in an internally rotated position. To maintain participant comfort, and reduce muscle activity during the scan, medical tape (Transpore™ Medical Tape, 3M™, USA) was wrapped around both feet to keep them in the required position through the scanning protocol (Figure 2.1). A default total-body scan (EF 8.4 lSv) was selected for all trials; scans emitted dual energy (140/100 kVp) fan-beam x-rays and lasted for ~7 minutes. The scanning region was 195 cm x 65 cm with 1.3 cm line spacing and a 0.2 cm point resolution. Each participant was exposed to ~8.4 μSv (Blake et al., 2006). Glickman et al. (2004) showed that DEXA gives a reliable measurement of total-body mass (r = 0.940), fat (r = 0.970) and lean mass (r = 0.890) against computer tomography in controls. Similar correlations are observed in obese comparisons to computer tomography with measures of trunk fat mass (r = 0.940), leg fat mass (r = 0.940) and leg FFM (r = 0.760) all being reliable (Bredella et al., 2010). In addition, the interrater reliability of DEXA scanning has been shown to be in excess of 0.998 (Hart et al., 2015).

2.3.3 Segmental definitions

Following DEXA scanning, each scan was split into 15 segments (Figure 2.2) using descriptions by Dempster (1955). Central segments were defined as: Head and Neck (HaN); thorax and; pelvis. The appendicular skeleton was segmented into and defined as: upper arm (UA); forearm (FA); hand; thigh; shank, and; foot. In addition, a secondary segmentation was conducted with the HaN, thorax and pelvis combined (HTP) and the left and right limbs being summed such that total-arm was the sum of UA, FA and hand, while the total-leg was the sum of thigh, shank and foot. Analysis of groups' body composition of the total-body was conducted post scan with segmental analyses conducted based on previous methods (Durkin et al., 2002; Durkin and Dowling, 2003). Digitisation of scans was completed using Physician's View v6.1 software (Hologic, UK) with segments separated using a series of squares, rhomboids and pentagons along the transverse axis of each respective joint (Figure 2.2).

Figure 2.1: An example of the participant set up for a total-body DEXA scan.

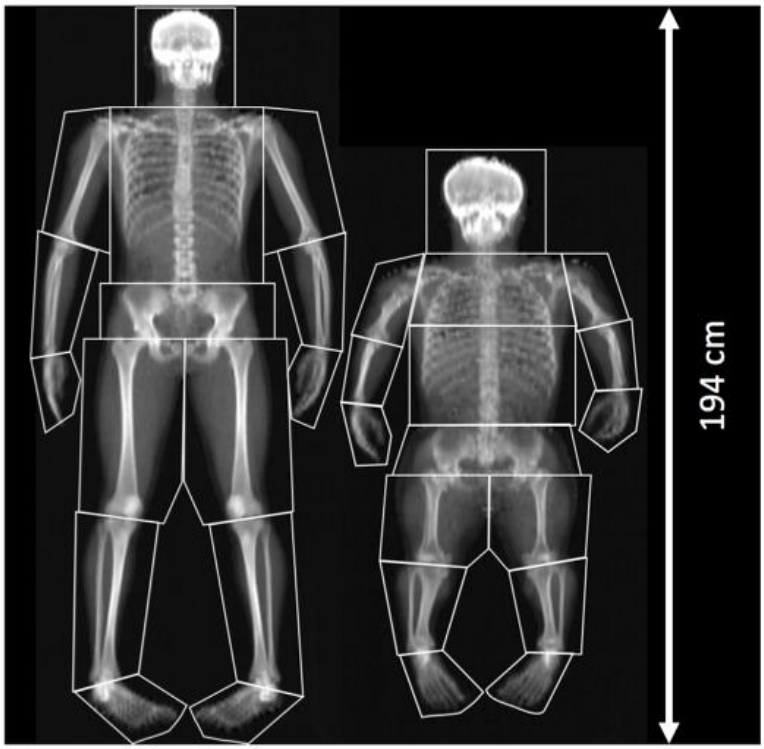

Figure 2.2: DEXA scans for (left) control and (right) an individual with Achondroplasia after segmental analyse. Note: the thorax of Achondroplasia was divided into two segments and then summed after analysis to ensure the correct mass was encompassed in the region.

2.3.4 Volumetric analysis of BMD

Post DEXA scan analysis was also used to segment the lumbar region of the spine (superior transverse plane of L1 to inferior transverse plane of L4, defined as L1-L4) using digitising software (Image J, National Institute of Health, Version 1.03i) to estimate the BMC of L1-L4 (BMC_{LUM}). Two of the 10 participants with Achondroplasia vertebral column were not identifiable post scan and so were omitted from this analysis. The lumbar vertebral column (L1-4) was assumed cylindrical and reliable methods (R = 0.979-0.992) previously described (Kröger et al., 1992; Sabin et al., 1995; Ott et al., 1997) were then used to measure the width of the lumbar vertebral column and its BMC. BMD_{VOL} was then calculated as:

$$Equation\ 2.1: Lum_{VOL} = \pi \cdot r^2 \cdot H$$

$$Equation\ 2.2: BMD_{VOL} = \frac{BMC_{LUM}}{Lum_{VOL}}$$

Where Lum_{VOL} is the volume of the lumbar (L1-L4) vertebrae, π is 3.14, r is the radius of the lumbar (i.e. half the width measured by ImageJ), H is lumbar column's height measured by ImageJ, BMC_{LUM} is the bone mineral content of the lumbar region measured by DEXA and BMD_{VOL} is the volumetric bone mineral density of the lumbar column (Figure 2.3).

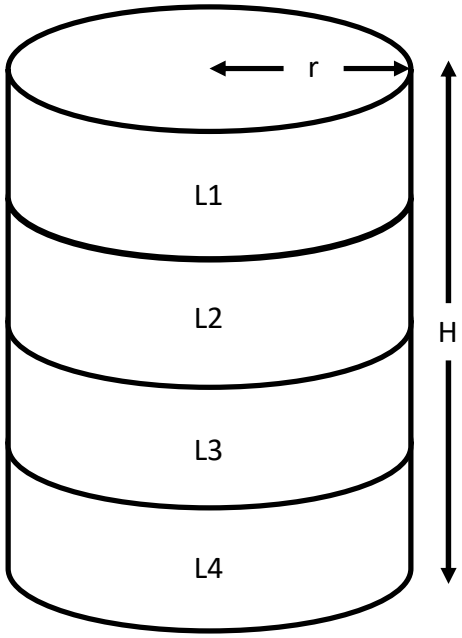

Figure 2.3: Volumetric calculations of BMD using DEXA data, adapted from Kröger et al. Where r is radius, H is the height of the lumbar column, L1-4 is each of the respective lumbar vertebrae.

2.3.5 Segment lengths

Following DEXA scans, participants underwent segmental length analysis in a separate air-conditioned motion analysis laboratory. 3-Dimensional motion analysis hardware (VICON, Oxford Metrics, UK) was used to determine the length of limbs and segments. Participants wore only shorts or tight-fitting clothing after anthropometric measures described by the User Manual were taken and entered into the software (Bodybuilder, 'plug-in-gait model', VICON Motion Systems, Oxford). Fourteen cameras were positioned on scaffolding which gave a ~170 m^3 viewing area. Calibration was completed following the manufacturers guidelines, such that the residual was < 0.01 mm. The model used to determine limb length was

Plug-in-Gait, which used thirty-nine 5 mm diameter reflective markers to determine limb lengths from joints centre predictions (see Figure A2.1 and Table A2.1 in Appendix 2 for marker placement details). Hip joint centres were calculated using previous equations (Davis et al., 1991) while all other joint centres were predicted based on the anthropometric measure described in the User Manual.

2.3.6 Statistical analysis

All data were collated on a personal computer (Macintosh, California) and inferentially analysed using SPSS (v22.0, IBM). The scan of each participant was analysed twice, over the period of one week, to assess the reliability of total-body mass and segment mass. To determine the intra-rater reliability a Bland-Altman limits of agreement plot and coefficient of variation was conducted to ascertain the agreement of the segmental analysis (Bland and Altman, 1986). Paired sampled t-tests were used for Day-1 (D1) and Day-2 (D2) segmental comparisons; intra-class correlation coefficient (ICC) was used to assess the intra-rater reliability of total-body mass and segment mass for the group with Achondroplasia and controls, as total-segment mass was deemed adequate to validate other coefficients given by DEXA (Glickman et al., 2004). All segment lengths and body composition variables data were initially compared between groups as a total-body measure. Following, each individual segment was presented relative to total-body values (%) and relative to its respective limb (%) as described in the segmental definitions section of the method. A multivariate analysis of variance (MANOVA) was used to establish differences between left and right sides and between groups. Independent t-tests were carried

out on total-body, central segments and lumbar BMD_{VOL} values between groups. For variables that violated parametric assumptions, Mann-Whitney U tests were performed to assess between-group differences of the central segments, while data that violated Levene's test were corrected using the non-equal variance option in SPSS (Greenhouse Geisser). Alpha was set at ≤ 0.05 with all results reported as means (SD).

2.4 Results

2.4.1 Intra-rater reliability

Data for D1 and D2 in Achondroplasia and control are displayed in Table 2.2. ICCs ranged from 0.908 - 0.997 for segment and total masses (Table 2.2). Coefficient of variation ranged between 11.2 - 25.2% for Achondroplasia and 7.8 - 18.1% for control over all segments (Table 2.2). No systematic bias existed ($P > 0.05$) in either group for any segment suggesting that the measurement of mass in all segments using DEXA were agreeable and reliable.

2.4.2 Total-body composition

There was no difference in age between groups ($P = 0.487$, Table 2.1). The group with Achondroplasia were 23% smaller in stature ($P < 0.001$), had 19% less body mass ($P < 0.001$) and had a 25% greater BMI ($P < 0.001$) than controls (Table 2.1). They also had 15% less total-body BMD ($P < 0.001$), 32% less BMC ($P < 0.001$) and 26% less FFM ($P < 0.001$) than controls (Table 2.1). There was no difference in fat mass between

groups (P = 0.447), but the group with Achondroplasia group had a higher body fat percentage than controls (P < 0.001, Table 2.1).

Table 2.2: Total masses (kg) for segments taken in Day 1 and Day 2 for Achondroplasia and controls. Values displayed for Day 1 and Day 2 are mean and the average measure as mean (SD).

	Achondroplasia						Control					
	Day 1	Day 2	Bias %	Average meausure	ICC	CV	Day 1	Day 2	Bias %	Average meausure	ICC	CV
HaN	5.6	5.6	0.8	5.6 (0.4)	0.984 *	7.8	5.9	5.9	0.3	5.9 (0.7)	0.916 *	11.2
Thorax	25.1	25.0	0.1	25.0 (3.7)	0.984 *	14.7	23.6	23.0	2.4	23.3 (3.2)	0.991 *	13.6
Pelvis	8.3	8.3	0.0	8.3 (1.2)	0.964 *	14.4	7.8	8.2	-5.9	8.0 (1.5)	0.908 *	18.3
Upper Arm	2.5	2.5	-2.3	2.5 (0.4)	0.978 *	17.6	1.5	1.5	3.4	1.5 (0.3)	0.991 *	20.3
Fore Arm	1.2	1.2	-2.7	1.2 (0.2)	0.987 *	13.1	0.9	0.9	0.9	0.9 (0.1)	0.979 *	15.9
Hand	0.4	0.4	-3.3	0.4 (0.1)	0.900 *	10.6	0.3	0.4	-4.3	0.4 (0.0)	0.939 *	12.5
Thigh	6.6	6.7	-0.1	6.7 (1.8)	0.990 *	18.1	4.3	4.3	0.4	4.3 (1.1)	0.996 *	25.2
Shank	2.2	2.2	-2.7	2.2 (0.5)	0.975 *	14.8	1.5	1.5	0.7	1.5 (0.4)	0.997 *	23.6
Foot	0.6	0.7	-4.5	0.7 (0.1)	0.974 *	12.9	0.5	0.5	-0.7	0.5 (0.1)	0.950 *	15.7
HTP	39.0	38.9	0.2	38.9 (4.8)	0.997 *	12.4	37.3	37.1	0.3	37.2 (4.8)	0.991 *	13.0
Whole-arm	4.1	4.2	-1.9	4.2 (0.6)	0.994 *	15.2	2.7	2.7	1.6	2.7 (0.5)	0.992 *	17.0
Whole-leg	14.7	14.8	-0.9	14.7 (2.3)	0.995 *	15.6	9.8	9.9	-0.8	9.8 (1.4)	0.997 *	14.7

ICC, Intraclass correlation; CV, coefficient of variation for segment comparisons between Day 1 and Day 2 within each group. * $P \leq 0.001$ for ICCs

2.4.2.1 Segment Analysis

MANOVA showed no difference in any body composition measure between left and right limbs therefore, data are presented as mean values between left and right limbs. The total-mass of all segments in the group with Achondroplasia were lower than controls ($P < 0.001$) and there were significant effects between groups' BMC, BMD, FFM and body fat mass ($P < 0.001$, Table 2.3).

2.4.2.2 Segment Length

The lengths of the HaN, pelvis and HTP were not different between groups ($P = 0.636$; $P = 0.601$ and $P = 0.097$, respectively), but the group with Achondroplasia group had a shorter thorax ($P = 0.006$, Table 2.3). The group with Achondroplasia had a shorter UA ($P < 0.001$), FA ($P < 0.001$), thigh ($P < 0.001$), shank ($P < 0.001$) and foot ($P < 0.001$) compared to control, there was no difference in hand length between groups ($P = 0.893$, Table 2.3). The total-arm and total-leg length of the group with Achondroplasia were shorter than controls ($P < 0.001$ and $P < 0.001$, respectively, Table 2.3).

Relative to stature, the group with Achondroplasia had a longer HaN ($P < 0.001$), thorax ($P = 0.002$) pelvis ($P < 0.001$) and HTP than controls ($P < 0.001$, Table 2.4 and Figure 2.4). The group with Achondroplasia also had a shorter UA ($P < 0.001$), FA ($P < 0.001$), hand ($P = 0.021$), thigh ($P < 0.001$) and shank ($P < 0.001$) than controls when relative to stature (Table 2.4 and Figure 2.4). Conversely, the group with Achondroplasia had a longer foot relative to stature than control ($P < 0.001$, Table

2.4 and Figure 2.4). Relative to stature, the total-arm and total-leg lengths were shorter in the group with Achondroplasia than controls ($P < 0.001$ and $P < 0.001$ respectively, Table 2.4 and Figure 2.4).

Relative to HTP, the group with Achondroplasia had a longer head ($P = 0.010$) and shorter thorax ($P = 0.014$) than controls, but there was no difference in pelvis length between groups ($P = 0.538$, Table 2.5 and Figure 2.5). When relative to the total-arm length, the group with Achondroplasia had a shorter UA ($P < 0.001$) but longer hand ($P < 0.001$) than controls; there was no difference in FA between groups ($P = 0.133$, Table 2.5 and Figure 2.5). There was no difference in thigh or shank length between groups when relative to leg length ($P = 0.250$ and $P = 0.250$, respectively), but the group with Achondroplasia had a longer foot than controls ($P < 0.001$, Table 2.5 and Figure 2.5).

2.4.2.3 Segment Mass

There was no difference HaN ($P = 0.175$), thorax ($P = 0.230$), pelvis ($P = 0.393$) or HTP mass between groups ($P = 0.343$, Table 2.3). The group with Achondroplasia had less UA ($P < 0.001$), FA ($P < 0.001$), hand ($P < 0.001$) and total-arm mass than controls ($P < 0.001$, Table 2.3). The group with Achondroplasia also had less thigh ($P < 0.001$), shank ($P < 0.001$) foot ($P < 0.001$) and total-leg mass than controls ($P < 0.001$, Table 2.3).

Relative to TBM, the group with Achondroplasia had more HaN ($P < 0.001$), thorax (P

< 0.001), pelvis (P < 0.001) and HTP mass than controls (P < 0.001, Table 2.4 and Figure 2.4). Relative to the TBM, the group with Achondroplasia had less UA (P < 0.001), FA (P < 0.001) and total-arm mass than controls (P < 0.001), but no difference was found in hand mass between groups (P < 0.001, Table 2.4 and Figure 2.4). Relative to the TBM, the group with Achondroplasia had less thigh (P < 0.001), shank (P < 0.001) and total-leg mass than controls (P < 0.001), but there was no difference in foot mass between groups (P < 0.001, Table 2.4 and Figure 2.4).

Relative to HTP mass, the group with Achondroplasia had more HaN (P = 0.032) and less thorax mass than controls (P = 0.023, Table 2.5 and Figure 2.5). There was no difference in pelvis mass relative to HTP between groups (P = 0.300). Relative to the total-arm mass, the group with Achondroplasia had less UA than controls (P < 0.001), but more FA and hand mass (P < 0.001 and P < 0.001 respectively, Table 2.5 and Figure 2.5). There was no difference in thigh or shank mass relative to total-leg mass between groups (P = 0.204 and P = 0.477, respectively). The group with Achondroplasia had more foot mass relative to total-leg mass than controls (P < 0.001, Table 2.5 and Figure 2.5)

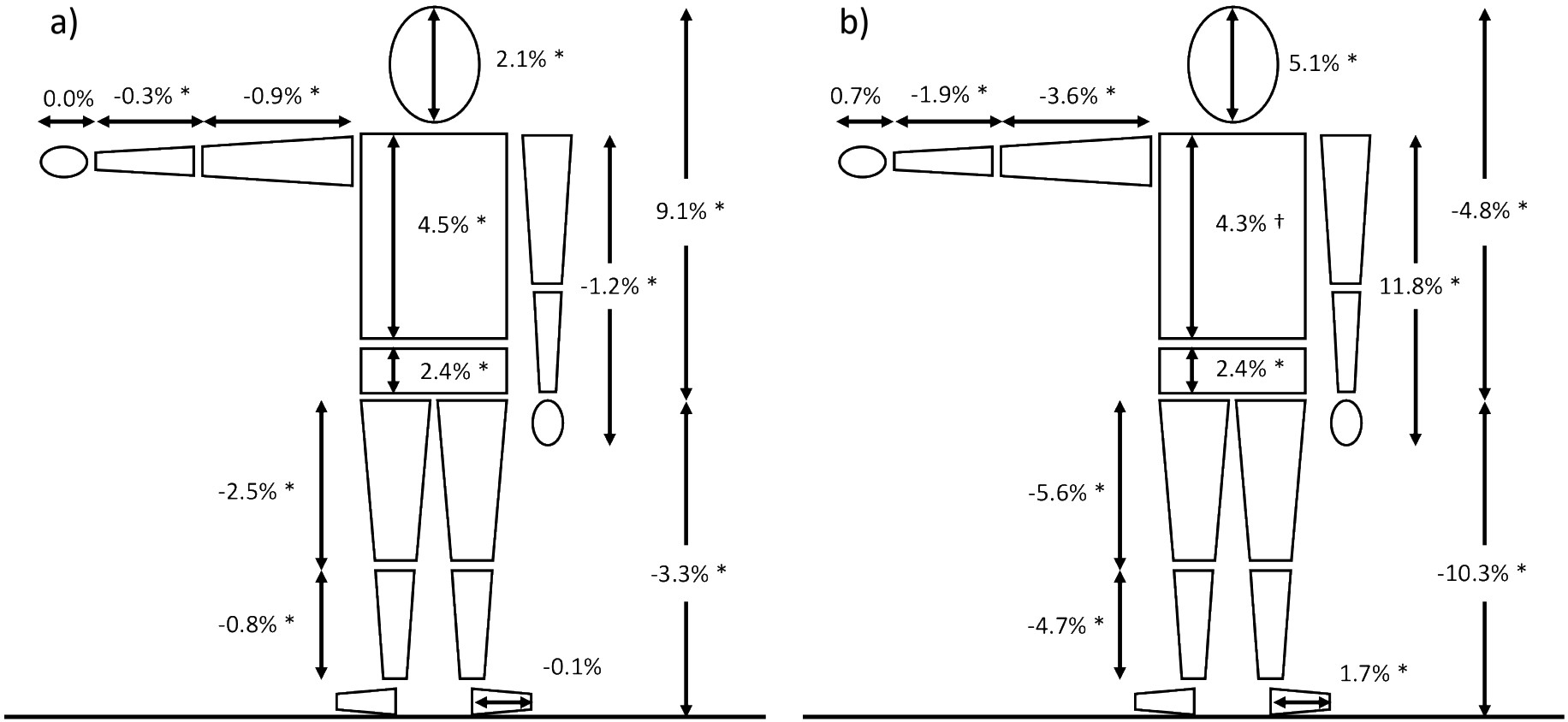

Figure 2.4: Mean percentage differences between the group with Achondroplasia *and control's a) segment length and b) segment mass relative to stature and total body mass respectively. Positive values represent a higher value in* the group with Achondroplasia *(Achondroplasia – control); † P < 0.01, * P < 0.001.*

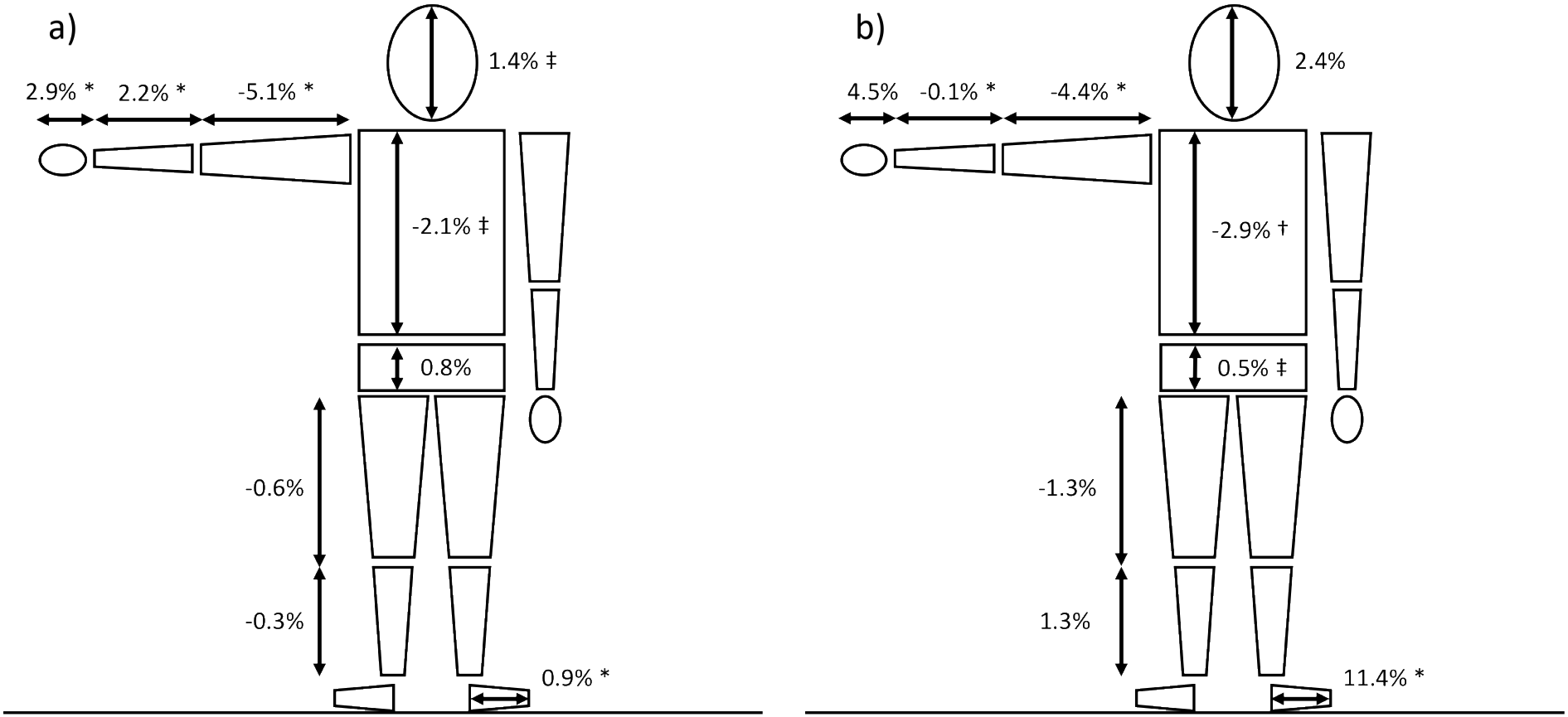

Figure 2.5: Mean percentage differences between the group with Achondroplasia *and control's a) segment length and b) segment mass relative to total-limb length and total-segment mass respectively. Positive values represent a higher value in* the group with Achondroplasia *(Achondroplasia – control); ‡ P < 0.05, † P < 0.01, * P < 0.001.*

2.4.3 Segment mass composition

2.4.3.1 Bone mineral content

There was no difference in BMC of the HaN (P = 0.415), thorax (P = 0.234) or HTP (P = 0.166) between groups. The group with Achondroplasia had less BMC than controls in the pelvis (P = 0.004), UA (P < 0.001), FA (P < 0.001), hand (P = 0.001), arm (P < 0.001), thigh (P < 0.001), shank (P < 0.001), foot (P < 0.001) and leg (P < 0.001, Table 2.3).

In the group with Achondroplasia, BMC relative to total-body BMC was higher in the HaN (P < 0.001), thorax (P < 0.001), HTP (P < 0.001), and hand compared to controls (P = 0.043). BMC relative to total-body BMC was not different between groups' pelvis (P = 0.342, Table 2.4). In the group with Achondroplasia, BMC relative to total-body BMC was lower in the UA (P < 0.001), FA (P < 0.001) total-arm (P < 0.001), thigh (P < 0.001), shank (P < 0.001), foot (P < 0.001) and total-leg compared to controls (P < 0.001, Table 2.4).

BMC relative to HTP was not different between groups in the thorax (P = 0.245) but was higher in HaN and pelvis in the group with Achondroplasia compared to controls (P = 0.004 and P = 0.002 respectively, Table 2.5). In the group with Achondroplasia, BMC relative to total-arm BMC was lower in the UA (P < 0.001), but higher in the hand compared to controls (P < 0.001, Table 2.5). Relative to total-leg BMC, the the group with Achondroplasia had a lower thigh BMC (P < 0.001) but a higher shank and foot BMC than controls (P < 0.001 and P < 0.001 respectively, Table 2.5).

2.4.3.2 Bone mineral density

There was no difference in HaN or thorax BMD between groups (P = 0.526 and P = 0.190, respectively). The group with Achondroplasia had lower BMD of the UA (P < 0.001), FA (P = 0.004), hand (P = 0.002), total-arm (P < 0.001), pelvis (P = 0.047), thigh (P < 0.001), shank (P < 0.001), foot (P < 0.001) and total-leg compared to controls (P < 0.001, Table 2.3). There was no difference in BMD of HTP between groups (P = 0.546, Table 2.3).

Relative to total-body BMD, the group with Achondroplasia had a higher BMD of the HaN (P = 0.004), thorax (P = 0.011) and HTP (P < 0.001) than controls, but no differences were observed between groups' pelvis (P = 0.822), UA (P = 0.634), FA (P = 0.141), hand (P = 0.812) or total-arm (P = 0.432, Table 2.4). The group with Achondroplasia had a lower BMD relative to total-body BMD, in the thigh (P < 0.001), shank (P = 0.005), foot (P = 0.001) and total-leg compared to controls (P < 0.001, Table 2.4).

Relative to HTP BMD, there was no difference in thorax BMD between groups (P = 0.637), but the group with Achondroplasia had a higher HaN (P = 0.039) and a lower pelvis BMD than controls (P < 0.001, Table 2.5). Relative to total-arm BMD, there was no difference in UA (P = 0.485), FA (P = 0.155) or hand BMD between groups (P = 0.668, Table 2.5). Relative to total-leg BMD, the group with Achondroplasia had a lower BMD of the thigh (P = 0.002), but a higher shank BMD than controls (P = 0.011, Table 2.5). No difference in foot BMD relative to total-leg BMD existed between groups (P = 0.857, Table 2.5).

2.4.3.3 volumetric BMD

The group with Achondroplasia had 31% less BMC_{LUM} compared to controls (P = 0.001). There was no group difference in BMD_{VOL} between groups (P = 0.597, Table 2.1).

2.4.3.4 Fat free mass

There was no difference in HaN or pelvis FFM between groups (P = 0.217 and P = 0.365, respectively), but the group with Achondroplasia had less thorax and HTP FFM than controls (P = 0.005 and P = 0.013 respectively, Table 2.3). The group with Achondroplasia had less UA (P < 0.001), FA (P < 0.001), hand (P < 0.001) and total-arm FFM than controls (P < 0.001, Table 2.3). The group with Achondroplasia also had less thigh (P < 0.001), shank (P < 0.001), foot (P < 0.001) and total-leg FFM than controls (P < 0.001, Table 2.3).

Relative to total-body FFM, the group with Achondroplasia had more HaN (P < 0.001), thorax (P < 0.001), pelvis (P < 0.001) and HTP FFM than controls (P < 0.001, Table 2.4). Relative to total-body FFM, the group with Achondroplasia has less FFM of all appendicular segments compared to controls (P < 0.05) other than hand and foot when no differences between groups were observed (P = 0.125 and P = 0.022, respectively, Table 2.4).

Relative to HTP FFM, the group with Achondroplasia had more HaN and less thorax FFM than controls (P < 0.001 and P = 0.027, respectively); no difference in pelvis FFM

between groups was observed (P = 0.894). Relative to total-arm FFM, the group with Achondroplasia had less UA and hand FFM (P < 0.001 and P < 0.001, respectively), but more FA FFM compared to controls (P < 0.001, Table 2.5). Relative to total-leg FFM, there was no difference in thigh or shank FFM between groups (P = 0.910 and P = 0.388, respectively), but the group with Achondroplasia had more foot FFM than controls (P = 0.008, Table 2.5).

2.4.3.5 Body fat mass

There was no difference in absolute body fat mass between groups in any segment (P > 0.05, Table 2.3).

When relative to total-body fat mass, there was no difference between groups' HaN or thorax fat (P = 0.524 and P = 0.061, respectively), but the group with Achondroplasia had more pelvis and HTP fat compared to controls (P = 0.013 and P = 0.017, respectively, Table 2.4). There was no difference in FA (P = 0.299), hand (P = 0.323) or total-arm fat (P = 0.431) when relative to total-body fat, but the group with Achondroplasia had more UA fat than controls (P = 0.025, Table 2.4). When relative to total-body fat, the group with Achondroplasia had less thigh (P = 0.003), shank (P = 0.034) and total-leg fat than controls (P = 0.001); there was no difference in groups' foot fat (P = 0.865, Table 2.4).

There were no differences in groups' HaN, thorax or pelvis fat when relative to HTP fat (P > 0.05, Table 2.5). Relative to total-arm fat, the group with Achondroplasia had

less UA and FA fat compared to controls (P = 0.003 and P = 0.013, respectively); no difference in hand fat was observed (P = 0.066, Table 2.5). There was no difference in thigh (P = 0.770), shank (P = 0.847) or foot fat between groups when relative to total-leg fat (P = 0.053, Table 2.5).

Table 2.3: Total-segment length, mass, BMC, BMD, FFM and fat for the group with Achondroplasia and controls. Values displayed as means (SD).

	Length (m)		Mass (kg)		BMC (kg)		BMD ($g \cdot cm^{-2}$)		FFM (kg)		Body Fat (kg)	
	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control
HaN	0.29 (0.03)	0.29 (0.08)	5.86 (0.70)	5.57 (0.40)	0.62 (0.08)	0.59 (0.08)	2.11 (0.21)	2.17 (0.25)	3.92 (0.49)	3.73 (0.28)	1.33 (0.16)	1.24 (0.09)
Thorax	0.42 (0.05) †	0.47 (0.03)	23.05 (3.12)	25.02 (3.55)	0.42 (0.11)	0.47 (0.07)	1.73 (1.02)	1.32 (0.59)	16.39 (1.89) †	19.43 (2.48)	6.18 (1.80)	5.09 (1.89)
Pelvis	0.16 (0.01)	0.16 (0.02)	8.24 (1.39)	8.32 (1.38)	0.20 (0.06) †	0.28 (0.06)	1.15 (0.37) ‡	1.37 (0.18)	5.34 (0.89)	6.05 (1.32)	2.33 (0.51)	1.91 (0.60)
UA	0.18 (0.02) *	0.30 (0.02)	1.48 (0.31) *	2.54 (0.44)	0.05 (0.01) *	0.10 (0.02)	1.00 (0.09) *	1.19 (0.14)	0.86 (0.20) *	1.83 (0.31)	0.57 (0.14)	0.58 (0.22)
FA	0.17 (0.02) *	0.26 (0.01)	0.85 (0.13) *	1.25 (0.17)	0.05 (0.01) *	0.08 (0.02)	0.94 (0.14) †	1.07 (0.15)	0.58 (0.09) *	0.96 (0.13)	0.23 (0.06)	0.21 (0.08)
Hand	0.09 (0.01) *	0.10 (0.02)	0.36 (0.04) *	0.45 (0.05)	0.02 (0.01) †	0.03 (0.01)	0.54 (0.09) †	0.64 (0.13)	0.22 (0.04) *	0.31 (0.05)	0.12 (0.02)	0.10 (0.05)
Thigh	0.25 (0.03) *	0.43 (0.02)	7.08 (1.14) *	10.71 (1.80)	0.13 (0.04) *	0.36 (0.07)	1.18 (0.11) *	1.72 (0.19)	4.45 (0.84) *	7.50 (1.55)	2.35 (0.49)	2.46 (0.84)
Shank	0.27 (0.04) *	0.43 (0.03)	2.11 (0.34) *	3.20 (0.52)	0.12 (0.02) *	0.24 (0.04)	1.04 (0.08) *	1.37 (0.18)	1.25 (0.16) *	2.19 (0.32)	0.74 (0.19)	0.77 (0.30)
Foot	0.19 (0.03) *	0.22 (0.01)	0.69 (0.07) *	0.90 (0.12)	0.04 (0.01) *	0.07 (0.02)	0.79 (0.09) *	1.11 (0.21)	0.46 (0.05) *	0.64 (0.09)	0.20 (0.04)	0.18 (0.06)
HTP	0.86 (0.07)	0.91 (0.05)	37.15 (4.87)	38.91 (4.76)	1.24 (0.20)	1.34 (0.16)	4.99 (1.46)	4.86 (0.75)	25.64 (3.13) ‡	29.21 (3.49)	9.83 (9.83)	8.25 (2.46)
Total-arm	0.45 (0.03) *	0.66 (0.04)	2.69 (0.46) *	4.23 (0.64)	0.12 (0.02) *	0.21 (0.04)	2.47 (0.28) *	2.90 (0.35)	1.66 (0.31) *	3.11 (0.45)	0.92 (0.19)	0.89 (0.32)
Total-leg	0.52 (0.03) *	0.86 (0.05)	9.88 (1.5) *	14.81 (2.34)	0.29 (0.05) *	0.68 (0.13)	3.00 (0.24) *	4.21 (0.46)	6.15 (0.96) *	10.33 (1.73)	3.29 (0.69)	3.41 (1.11)

Achon, Achondroplasia; HaN, Head and Neck; UA, Upper Arm; FA, Fore Arm; HTP, HaN, Torso and Pelvis; ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.

Table 2.4: Table 2.4: Total-segment length, mass, BMC, BMD, FFM and fat relative to total-body values for the group with Achondroplasia and controls. Values displayed as means (SD).

	Length (%)		Mass (%)		BMC (%)		BMD (%)		FFM (%)		Body Fat (%)	
	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control
HaN	21.0 (1.8) *	15.9 (0.1)	9.4 (0.7) *	7.3 (0.9)	30.4 (2.8) *	19.3 (3.3)	13.3 (1.0) †	11.5 (1.7)	9.5 (0.5) *	6.7 (0.9)	7.4 (1.2)	7.9 (2.0)
Thorax	30.3 (3.1) †	26.0 (1.2)	37.0 (1.3) *	32.5 (1.7)	20.3 (2.9) *	15.1 (1.2)	10.5 (4.7) ‡	6.7 (2.5)	39.8 (1.7) *	34.8 (3.0)	33.5 (4.0)	30.0 (4.6)
Pelvis	11.3 (0.8) *	8.9 (1.1)	13.2 (1.2) *	10.8 (0.9)	9.5 (2.3)	8.9 (1.1)	7.1 (1.5)	7.2 (0.7)	12.9 (1.2) *	10.7 (1.6)	12.8 (1.6)	11.3 (1.2)
UA	13.1 (1.1) *	16.7 (0.6)	2.4 (0.3) *	3.3 (0.3)	2.4 (0.3) *	3.2 (0.3)	6.3 (0.6)	6.2 (0.4)	2.1 (0.3) *	3.3 (0.4)	3.1 (0.4)	3.4 (0.4)
FA	12.6 (1.0) *	14.6 (0.5)	1.4 (0.1) *	1.6 (0.1)	2.4 (0.3) ‡	2.6 (0.5)	5.9 (0.8)	5.6 (0.7)	1.4 (0.1) *	1.7 (0.2)	1.3 (0.2)	1.2 (0.2)
Hand	6.3 (1.2)	5.6 (1.0)	0.6 (0.1)	0.6 (0.1)	1.1 (0.3) ‡	1.0 (0.2)	3.4 (0.6)	3.4 (0.6)	0.5 (0.1)	0.6 (0.1)	0.7 (0.1)	0.6 (0.2)
Thigh	18.4 (2.8) *	24.1 (1.1)	11.4 (0.7) *	13.9 (0.6)	6.4 (1.8) *	11.5 (1.1)	7.4 (0.8) *	9.0 (0.5)	10.8 (1.6) *	13.3 (1.6)	12.9 (1.1)	14.6 (2.2)
Shank	19.4 (2.2) *	24.1 (0.9)	3.4 (0.3) *	4.2 (0.3)	5.7 (0.6) *	7.7 (0.6)	6.5 (0.6) †	7.2 (0.9)	3.0 (0.3) *	3.9 (0.6)	4.1 (0.6)	4.5 (0.8)
Foot	13.9 (2.3) *	12.2 (0.6)	1.1 (0.1)	1.2 (0.2)	1.9 (0.3) *	2.4 (0.4)	5.0 (0.6) †	5.8 (0.9)	1.1 (0.1)	1.2 (0.2)	1.1 (0.2)	1.1 (0.3)
HTP	62.6 (3.4) *	50.8 (1.7)	59.7 (1.4) *	50.6 (1.6)	60.2 (3.1) *	43.2 (3.5)	30.9 (5.4) *	25.4 (2.3)	62.2 (2.5) *	52.2 (2.2)	53.7 (53.7)	49.2 (5.0)
Total-arm	32.5 (2.3) *	36.9 (1.1)	4.3 (0.3) *	5.5 (0.4)	5.9 (0.7) *	6.9 (0.6)	15.6 (1.7)	15.2 (1.1)	4.0 (0.4) *	5.6 (0.5)	5.1 (0.5)	5.2 (0.6)
Total-leg	37.8 (2.4) *	48.1 (1.3)	15.9 (0.9) *	19.2 (0.8)	14 (1.9) *	21.5 (1.8)	18.9 (1.7) *	22.1 (1.6)	14.9 (1.7) *	18.3 (1.2)	18.1 (1.6)	20.2 (2.3)

Achon, Achondroplasia; HaN, Head and Neck; UA, Upper Arm; FA, Fore Arm; HTP, HaN, Torso and Pelvis; ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.

Table 2.5: Table 2.5: Total-segment length, mass, BMC, BMD, FFM and fat relative to total-limb values for the group with Achondroplasia and controls. Values displayed as means (SD).

	Length (%)		Mass (%)		BMC (%)		BMD (%)		FFM (%)		Body Fat (%)	
	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control
HaN	33.7 (2.8) †	31.3 (0.1)	15.8 (1.0) ‡	14.5 (1.7)	50.6 (5.2) *	44.4 (4.8)	44.1 (7.3) †	45.4 (7.1)	15.3 (0.9) *	12.9 (1.5)	13.9 (2.5)	16.1 (4.0)
Thorax	48.3 (3.3) ‡	51.1 (1.7)	62.1 (2.1) ‡	64.2 (2.3)	33.6 (3.4) *	34.9 (2.6)	32.8 (8.0) ‡	26.1 (7.6)	64.0 (1.5) *	66.5 (3.2)	62.2 (5.0)	60.7 (4.4)
Pelvis	18.1 (1.4)	17.6 (2.3)	22.1 (1.8)	21.3 (1.9)	15.8 (3.6)	20.6 (3.3)	23.1 (3.4)	28.5 (3.3)	20.7 (1.4) *	20.6 (3.3)	23.9 (3.2)	23.1 (2.3)
UA	40.6 (3.4) *	45.3 (1.5)	54.8 (2.6) *	59.8 (2.2)	41.2 (3.5) *	47.5 (5.3)	40.5 (2.9)	41.2 (3.4)	51.7 (3.3) *	58.8 (2.9)	61.7 (3.3)	64.9 (3.7)
FA	39.4 (3.3)	39.5 (1.8)	31.8 (1.8) *	29.6 (1.5)	39.8 (2.3) ‡	38.2 (6.3)	37.8 (1.7)	36.8 (2.6)	35.1 (2.9) *	31.1 (1.9)	25.3 (2.8)	23.4 (2.6)
Hand	19.7 (3.4)	15.2 (2.2)	13.5 (1.4) *	10.6 (1.1)	19.0 (3.1) ‡	14.3 (3.4)	21.7 (2.1)	22.0 (2.8)	13.3 (1.5)	10.1 (1.5)	13.0 (1.9)	11.7 (2.7)
Thigh	48.7 (6.1)	50.0 (1.5)	71.6 (1.7)	72.2 (1.7)	44.6 (10) *	53.4 (1.9)	39.2 (1.4) *	41.0 (2.3)	71.8 (4.2) *	72 (5.8)	71.6 (2.7)	72 (5.8)
Shank	51.3 (6.1)	50.0 (1.5)	21.3 (1.4)	21.6 (1.3)	41.5 (7.3) *	35.7 (1.7)	34.6 (1.4) †	32.7 (2.9)	20.6 (3.0) *	21.6 (4.5)	22.4 (2.4)	22.7 (6.1)
Foot	36.9 (6.6) *	25.5 (1.5)	7.1 (0.7) *	6.2 (0.9)	14.0 (3.0) *	10.9 (1.3)	26.2 (1.6) †	26.3 (2.8)	7.5 (1.4)	6.4 (1.5)	6.0 (0.7)	5.3 (1.4)

Achon, Achondroplasia; HaN, Head and Neck; UA, Upper Arm; FA, Fore Arm; HTP, HaN, Torso and Pelvis; ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.

2.5 Discussion

The aims of this study were to assess total-body, and segment, lengths and *in vivo* BMC, BMD, FFM and fat mass of adults with Achondroplasia and compare to controls. The main findings are that 1) adult males with Achondroplasia have shorter appendicular limbs and the same torso length to controls, 2) adults with Achondroplasia have less BMC, BMD and FFM than controls at the total-body and segmental level, but body fat mass was the same between groups, and 3) the differences in groups' segmental lengths and body composition are lessened and at times removed when relative to total-body and total-limb measures.

2.5.1 Segment lengths and masses

Achondroplasia is a medically defined condition which is characterised by an inhibition of growth plate activity during maturation and is commented on by many, in anecdotal terms, as 'rhizomelic' limb lengths (Ponseti, 1970; Horton et al., 1978b; Owen et al., 1990; Horton et al., 2007; Matsushita et al., 2016). However, there is extremely limited empirical evidence to support this description in adults with Achondroplasia. Contrary to previous anecdotal reports (Ponseti, 1970; Horton et al., 1978b; Hecht et al., 1988; Hunter et al., 1996a), absolute thorax length was significantly shorter in the current group with Achondroplasia compared to controls. When presented relative to stature, the thorax of the group with Achondroplasia was longer than controls, but no difference in HTP length was observed between groups. The data from the present study are similar to Nehme et al. (1976) and Owen et al. (1990) who measured torso length in individuals with Achondroplasia. Both observed

a shorter torso length (measured as sitting height) in their respective groups with Achondroplasia compared to controls, but Nehme et al.'s participants were aged between 0-18 years old (N = 11) while Owen et al. combined data from individuals with both Achondroplasia and Hypochondroplasia (a condition similar to Achondroplasia, but with milder symptoms). While the data from the present study appear to show that the torso of an individual with Achondroplasia is shorter than controls, when presented relative to the HTP, there are no differences in torso length between groups.

This study also showed that appendicular segments of individuals with Achondroplasia were shorter than controls and shorter relative to stature. These findings are again consistent with Nehme et al. (1976) and Owen et al. (1990). Nehme et al. (1976) measured the lower limb lengths of a group with Achondroplasia, but present their data as standard deviations, without inferential analysis. In addition, the measurements made by Nehme et al. (1976) were conducted using radiography imaging. Although valid, the position of any individual's body during imaging may have affected the perspective length of the limb. Owen et al. (1990) on the other hand used a measuring tape to record upper limb lengths of adults with Achondroplasia. As reported in the current Chapter, Owen et al. (1990) reported higher adiposity of their participants with Achondroplasia. It is likely that the measured limb length would have been larger in Owen's group with Achondroplasia due to the curvilinear shape of a segment with high adiposity. Given the large difference in absolute limb lengths between Nehme et al. (1976), Owen et al. (1990) and the current group with Achondroplasia compared to their respective controls,

the position of the body would undoubtedly not affect the overall findings of a 'shorter appendicular limb' compared to controls. The Euclidean measurement of limb length used in the current Chapter however, does allow for a more valid method of limb length measurement compared to those previously reported. Furthermore, the current study presents relative limb lengths of individuals with Achondroplasia which is unreported.

When relative to their respective total-limb length, few differences in segment lengths were observed between groups. This suggests that that the proportional growth of long bones is similar between group, albeit a severe stunting in absolute growth in individuals with Achondroplasia. A notable observation though was that the foot of individuals with Achondroplasia was longer (relative to leg length) than controls. This is similar to previous observations (Egginton et al., 2006) and may impact gait kinematics, which in turn may alter physiological and biomechanical variables, such as metabolic cost and joint power. However, very few reports have been made correlating limbs lengths and joint kinematics in populations with Achondroplasia to comment further.

Unsurprisingly, all absolute segment masses of individuals with Achondroplasia were less than controls. When presented relative to their respective total-segment masses though, differences between groups lessened. In some instances, the group with Achondroplasia had heavier segments; this is likely due to their larger amount of fat mass per segment compared to controls. Certainly, for the leg segments, there were no differences in groups thigh and shank mass, but the foot of the individuals with

Achondroplasia was relatively heavier (likely due to the additional length and possibly due to their higher BMC relative to total-leg BMC, discussed later). For functional measures, such as force production or walking, the thigh and shank appear to have similar amount of FFM which suggest that the same relative force could be produced by groups. This though, to the author's knowledge, does not appear to be measured in adults with Achondroplasia.

2.5.2 Bone mineral content and density

Bone density is associated with fracture risk in all populations (Marshall et al., 1996). As shown in this Chapter, the group with Achondroplasia had a lower total-body and individual segment BMC and BMD than controls. Similar total-body BMD results (Taşoğlu et al., 2014; Matsushita et al., 2016) and mandible and lumbar spine BMD have been observed in adults with Achondroplasia (Arita et al., 2013). The results in the Chapter are only comparable to a few participants included in those studies though due to the participant demographics and classifications of the participants' skeletal dysplasia used in all studies. In the present Chapter, the lower absolute BMC of the appendicular segments in the group with Achondroplasia suggest that the mutated FGFR3 gene not only results in shorter 'long' bones but may impact BMC within the bones. The lower BMD in the group with Achondroplasia is unsurprising given that the mutated FGFR3 gene results in shorter bones (Deng et al., 1996) and therefore less viewable area when DEXA scanning resulting in an under prediction of long bone's BMD (Bianchi, 2007). This may be the case in the current study as total-body and segmental values of BMC and BMD were lower than controls, to the point

where the group with Achondroplasia had a total-body BMD Z-score of -1.82. This would classify the group with osteopenia and at a 'higher risk' of bone fractures using the controls as reference data (French et al., 2002). Using total-body BMD to compare bone quality between groups of different limb length proportions, such as individuals with Achondroplasia, may lead to a misinterpretation of clinical state. In the current Chapter, BMC and BMD were made relative to total-body and total-limb masses to allow for a more informed comparison between groups. Scaling BMC and BMD did appear to remove statistical differences between groups.

The BMD_{VOL} was similar between groups despite the lower BMC of the group with Achondroplasia. Similar results of BMD_{VOL} are observed in the literature when different sized vertebra are compared (Kröger et al., 1995; Lu et al., 1996). When relative to total-body values, the differences in groups' BMC and BMD remained, but when relative to total-limb, differences between groups' BMC and BMD values are removed and, at times, reverse. For example, in some segments, BMC was lower in the group with Achondroplasia than controls when relative to total-body BMC, suggesting that the mutated FGFR3 gene that causes Achondroplasia indeed alters the bone structure and quality rather than just the end plates (Horton and Lunstrum, 2002). However, when the shank and foot BMC and BMD values was presented relative to their respective total-leg BMC and BMD values, the group with Achondroplasia had a higher BMC and BMD than controls. It is possible that these results are due to a higher magnitude of force and a more frequent application of force during activities of daily living, such as walking, for individuals with Achondroplasia than controls. This can partially be explained by the presented

results as the group with Achondroplasia have a greater upper body mass relative to total-body mass than controls. Therefore, the body weights (ground reaction force ÷ mass) experienced by the smaller foot and shank of individuals with Achondroplasia is likely to be higher than controls during walking and/or running. Such results are observed elsewhere in populations where mass distribution is manipulated (Browning and Kram, 2007; Grabowski and Kram, 2008), but are unconfirmed in populations with Achondroplasia. Furthermore, the shorter legs of individuals with Achondroplasia are likely to lead to a higher stride frequency at habitual walking speeds than controls, like that observed between groups of different stature (Minetti et al., 1994; Schepens et al., 2004). The higher relative ground reaction force and greater loading frequency would likely increase the bone turnover of individuals with Achondroplasia, leading to a higher BMC and BMD of their lower limb segments compared to controls. To back this theory in individuals with Achondroplasia though, either an *in vitro* analysis of their lower limb bones is required, or, a longitudinal analysis combining force development during activities, such as walking and/or running, and the monitoring of their BMC and BMD are required. Certainly, though a large population specific database is required for the BMC and BMD of individuals with Achondroplasia to be compared to allow clinicians to make informed classifications of their bone health.

2.5.2 Fat free mass and body fat

The lower total-body and total-limb FFM of the group with Achondroplasia seen here would be explained, in part, by their shorter limbs and their higher total-limb fat mass. Similar to the bone density measures, when the FFM and fat of individual segments were presented relative to total-limb values, differences in both FFM and fat were somewhat removed; only lower FFM and fat of the arm segments were apparent in the group with Achondroplasia. The composition of the leg segments' FFM within the group with Achondroplasia may therefore include the same relative contractile elements as controls and would likely produce the same amount of relative torque production as controls. This though, is yet to tested empirically.

In controls, BMI is a widely used measure of estimating body fat as the two variables correlate positively (Flegal et al., 2009). In the present Chapter, the BMI and body fat percentage of the group with Achondroplasia would class them as 'moderately obese' and place them 'at risk' of cardiovascular disease (Després et al., 1990; Després et al., 2006; Freedman et al., 2013; Rabkin, 2014; Kihara and Matsuzawa, 2015). Previous work by Hecht et al. (1987), and more recently Wynn et al. (2007), report high rates of cardiovascular disease attributed deaths in individuals with Achondroplasia (~32%). The data in this Chapter suggest the higher cardiovascular death rate this is attributed to the higher thorax and pelvis fat in the group with Achondroplasia, as higher levels of abdominal fat are associated with cardiovascular deaths in controls (Yusuf et al., 2004; Van Gaal et al., 2006). While the group with Achondroplasia have the same fat mass as controls in the thorax and pelvis, their masses relative to total-body values suggest a higher abdominal fat than controls

(Figure 2.6) and therefore a possible reason for the attributed cardiovascular deaths observed in the population. However, less HTP fat was observed in the individuals with Achondroplasia when presented relative to total-body fat, which contradicts the speculation above but is likely due to their relatively larger torso. To make any more substantial conclusions on this topic is difficult from the available data sets though. Further work investigating the longitudinal analyses of body fat and its distribution, diet and lifestyle (e.g. physical activity) is required in individuals with Achondroplasia.

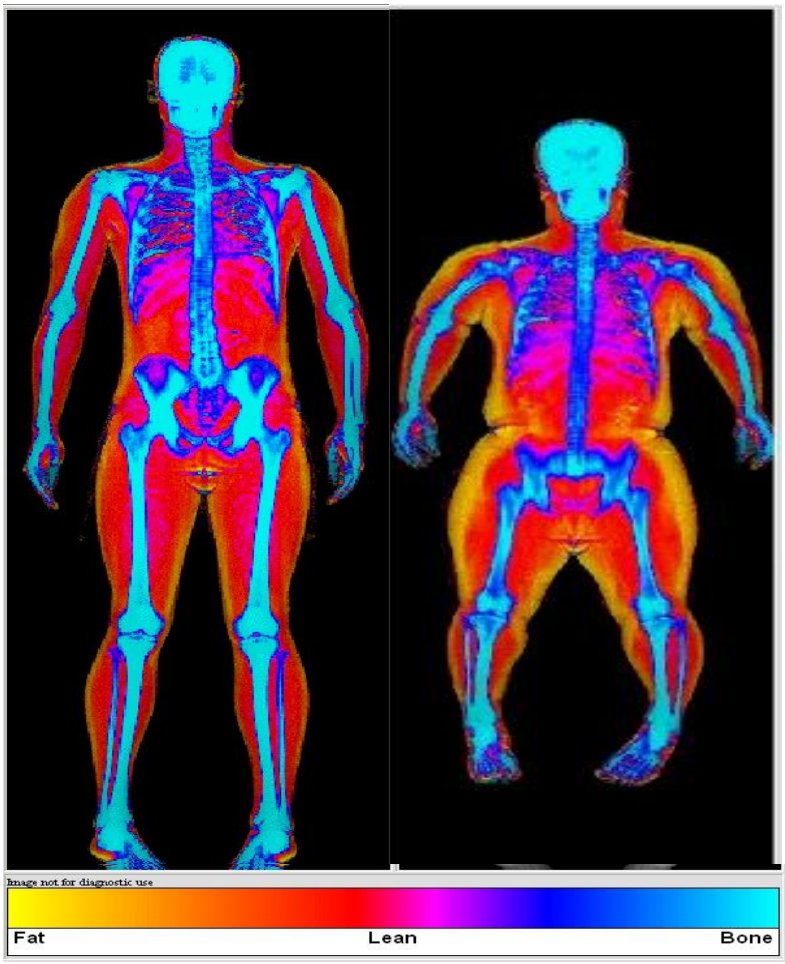

Figure 2.6: A DEXA scan of a control (left) and a male participant with Achondroplasia (right) showing the distribution of fat, lean and bone mass (see scale below image).

2.5.3 Clinical implications

Achondroplasia is irreversible, but the development of bone following surgical procedures of bones, such as leg lengthening appear normal (Venkatesh et al., 2009; Park et al., 2015). Certainly, from the presented BMC and BMD data of the shank and foot relative to the total-leg values, it would be assumed that bone turnover and development is similar to controls despite the mutated FGFR3 gene. Furthermore,

the current data suggest that an increased BMC of all bones could be achieved in populations with Achondroplasia through appropriate interventions. Loading and stressing of bone through exercise interventions have been shown to improve BMC of 'osteopenic' groups. For example, in the elderly, BMD of the femoral neck (Vincent and Braith, 2002; Beavers et al., 2017) and lumbar column (Beavers et al., 2017) increase following resistance and aerobic exercise interventions, respectively. It is likely that the BMC, BMD and muscle mass (here as FFM) would improve (i.e. become higher) in populations with Achondroplasia through such exercise interventions. However, to date, there appears to be no structured exercise intervention aimed at improving bone health or FFM in any population with Achondroplasia. With the condition affecting bone end plate development and structure, it is likely that the ability of a person with Achondroplasia performing complex resistance exercises is different to controls. Therefore, it would be advised that movement analyses of different exercises be explored in the populations with Achondroplasia prior to intervening with previously utilised exercise modes.

2.6 Conclusion

The aim of this study was to measure and compare body composition between adults with Achondroplasia and controls. The main findings of this study were total-body composition of the group with Achondroplasia suggested they were 'at risk' of a number of health complications, such as osteoporosis. Scaling the body composition of individual segments to the respective HTP and total-limb mass however, was appropriate to remove these classifications in the group with Achondroplasia.

Further work is required to create databases for the body compositions of populations with Achondroplasia to be compared to.

Chapter 3: The maximal oxygen uptake of adults with Achondroplasia

3.1 Abstract

Individuals of shorter stature generally display a reduced absolute maximal oxygen consumption ($\dot{V}O_{2max}$, L ·min^{-1}). The presentation of $\dot{V}O_{2max}$ relative to total-body mass (TBM) or fat free mass (FFM), removes statistical differences in $\dot{V}O_{2max}$ between different shorter and taller groups. In addition, physiological variables associated with $\dot{V}O_{2max}$ may also help predict submaximal oxygen consumption ($\dot{V}O_2$) without the need to perform a $\dot{V}O_{2max}$ test. The aim of this study was to 1) measure $\dot{V}O_{2max}$ in adult males with Achondroplasia (N = 10, age 22 ±3 yrs) and age matched adults of average stature (controls, N = 17, age 22 ±2 yrs) and 2) observe any trends between $\dot{V}O_{2max}$ and other physiological variables. Indirect calorimetry was used to measure $\dot{V}O_{2max}$ during an incremental treadmill test to volitional exhaustion. Heart rate (HR), minute ventilation (V_E), tidal volume (V_T), breathing frequency (B_f) and respiratory exchange ratio (RER) were also collected throughout the exercise protocol and correlated with $\dot{V}O_2$. The group with Achondroplasia had a 24% lower absolute $\dot{V}O_{2max}$ than to controls (P = 0.002). There was no difference between groups' $\dot{V}O_{2max}$ when relative to TBM or FFM (P > 0.05). Positive trends existed between percentage of $\dot{V}O_{2max}$ and HR, and V_E respectively, for both groups (r = 0.977 – 0.995, P < 0.001). These data suggest that $\dot{V}O_{2max}$ relative to TBM and FFM are the same in between individuals with Achondroplasia and controls. Therefore, previously advocated exercise programmes used in controls, can be utilised in individuals with Achondroplasia.

Key Words: Achondroplasia; Maximal oxygen consumption; Fat Free Mass

3.2 Introduction

As reported in Chapter 2, Achondroplasia is a condition characterised by shorter stature manifested by a disproportionate limb-to-torso length compared to age matched individuals of average stature (controls). In groups of shorter stature, a reduced absolute maximal oxygen consumption ($\dot{V}O_{2max}$) is observed compared to taller groups (Ferretti et al., 1991; Cuneo et al., 1991; Woodhouse et al., 1999; Takken et al., 2007). There is however, a positive correlation between stature and mass and therefore the reduction of $\dot{V}O_{2max}$ in the shorter population is likely due to a lower total-body mass (TBM). For individuals with Achondroplasia, mass and height are disproportionate. Their shorter stature may therefore infer a lower $\dot{V}O_{2max}$, but their disproportionate mass to stature ratio may skew relative presentations of $\dot{V}O_{2max}$. The available $\dot{V}O_{2max}$ data in populations with Achondroplasia is limited to one group of children and shows a lower $\dot{V}O_{2max}$ than controls when presented absolutely and relative to TBM (Takken et al., 2007). There are however no $\dot{V}O_{2max}$ data available in adult populations with Achondroplasia to help confirm this report.

Exercise performance, health status and mortality can be predicated from the accurate measurement of $\dot{V}O_{2max}$ (Kodama et al., 2009). There is a high rate of cardiovascular related deaths in adults with Achondroplasia (Wynn et al., 2007; Hecht et al., 1987), which may be attributed to a lower $\dot{V}O_{2max}$ compared to controls, but is unsubstantiated. An accurate prediction of $\dot{V}O_{2max}$ is therefore imperative to understand any causative links to mortality within the group. Given the disproportionate mass-to-stature ratio of individuals with Achondroplasia, and their

apparent higher adiposity (Chapter 2), presenting their $\dot{V}O_{2max}$ relative to TBM may under-predict their $\dot{V}O_{2max}$ and therefore misrepresent their health status. As mammalian skeletal muscle is reported to utilise ~90% of the consumed oxygen during exercise (Lolli et al., 2017), and assuming fat free mass (FFM) more accurately represents muscle mass than TBM, presenting $\dot{V}O_{2max}$ relative to FFM may be more appropriate for individuals with Achondroplasia. Indeed for obese groups, presenting $\dot{V}O_{2max}$ relative to FFM lessens the difference in absolute $\dot{V}O_{2max}$ compared to lean groups (Goran et al., 2000; Lolli et al., 2017). In the reported child group with Achondroplasia, $\dot{V}O_{2max}$ relative to TBM is 44% lower than controls (Takken et al., 2007). Were FFM used to present $\dot{V}O_{2max}$ in this group, instead of TBM, some of this difference may have been removed, leading to a more accurate representation of the group's $\dot{V}O_{2max}$. To the author's knowledge though, no such method has been made in any population with Achondroplasia.

While $\dot{V}O_{2max}$ is useful to describe and predict health status, exercise capacity, and mortality, it also affords the ability to help predict exercise intensities that can be used for cardiovascular training. For example, steady state exercise is commonly prescribed based on $\dot{V}O_{2max}$ or maximal heart rate (HR_{max}) values, as the two variables correlate positively during graded exercise in both lean and obese people (W. C. Miller et al., 1993; Strath et al., 2000). Physiological responses, such as training thresholds or oxidation of substrates, can be then be estimated based on percentages of $\dot{V}O_{2max}$ or HR_{max} values (Achten and Jeukendrup, 2004; Helgerud et al., 2007). For adults with Achondroplasia though, there appears to be no data that indicate if physiological variables, such as oxygen uptake ($\dot{V}O_2$) and HR correlate.

Training programmes that are designed for individuals with Achondroplasia that are based on current methods may therefore be misleading.

The aims of this study were therefore to describe the $\dot{V}O_{2max}$ of adults with Achondroplasia and compare them to controls. The primary objectives were to:

1) collect $\dot{V}O_{2max}$ in adults with Achondroplasia and compare to controls;
2) attempt to account for any differences in absolute $\dot{V}O_{2max}$ by presenting values relative to TBM and FFM;
3) observe if $\dot{V}O_2$ correlates with physiological variables (namely HR) similarly in both groups.

It was hypothesised that 1) the group with Achondroplasia would have a lower absolute $\dot{V}O_{2max}$, but differences would be reduced when presented relative to TBM and FFM; and, 2) $\dot{V}O_2$ would positively correlate with HR, but the similarity to controls is unknown.

3.3 Methods

3.3.1 Participants and general procedure

Ten adults with Achondroplasia and 17 age matched controls that were free from lower limb injury volunteered to participate in the study and are described in Table 2.1 in Chapter 2. All were experienced in treadmill running.

3.3.2 Anthropometric measures

Participants' TBM (kg) was obtained using electronic scales (SECA 813, CA 91710 Chino, USA) while barefooted and wearing minimal clothing. FFM was obtained using Dual energy x-ray absorptiometry (DEXA), described in detail in section 2.3.2 of Chapter 2.

3.3.3 Collection of oxygen consumption

Expired gases, minute ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (B_f) and respiratory exchange ratio (RER) were collected and analysed using portable breath-by-breath indirect calorimetry (Metamax 3B, Cortex, Leipzig Germany), which was calibrated to the manufacturer's guidelines prior to testing. The portable indirect calorimeter (weight = 1 kg) and a fitted face mask (Hans Rudolph V2, dead space between 125 – 143 ml) were worn by participants during the exercise bout (see Figure 3.1).

3.3.4 Exercise protocol

While wearing the above apparatus, a 5-minute warm up which consisted of a self-selected jogging speed and self-prescribed light stretches was conducted by all participants prior to testing. Participants were also familiarised with treadmill running while wearing the portable calorimeter and facemask. To attain the speed at which $\dot{V}O_{2max}$ would be obtained by all participants, a steady rate step protocol was used. Participants started running on a motor driven treadmill (Woodway PPS70) at 6 kph with a speed increment of 1 kph every 3 minutes added until the third stage

(8 kph) was completed. Both HR (Polar) and $\dot{V}O_2$ were recorded throughout the $\dot{V}O_{2max}$ test. The estimated running speed from which $\dot{V}O_{2max}$ would be achieved was obtained by extrapolating HR data to age predicted maximal HR (HR_{max}) (Achondroplasia 8.8 ±0.9 kph; controls 12.4 ±1.4 kph). A ramped protocol was then used to attain $\dot{V}O_{2max}$ where by participants ran at their respective extrapolated treadmill speed whilst treadmill incline increased 1%·min^{-1} from 1% until volitional exhaustion (Porszasz et al., 2003). $\dot{V}O_2$ data were analysed following the completion (MetaSoft®, Cortex, Leipzig Germany) of the exercise with $\dot{V}O_{2max}$ identified as a plateau in the $\dot{V}O_2$ trace and met criteria described previously (Poole et al., 2008). Based on criteria for establishing $\dot{V}O_{2max}$ (Poole et al., 2008) an observable plateau in $\dot{V}O_2$, RER >1.15 and HR_{max} within 10 bpm of age predicted maximum were considered with $\dot{V}O_{2max}$ being recorded as a rolling average of 10 seconds either side of the peak $\dot{V}O_2$ curve, an example is given in Figure 3.2. HR, $\dot{V}_E$, V_T, B_f and RER were also measured throughout the $\dot{V}O_{2max}$ protocol with values taken over the same period $\dot{V}O_{2max}$ was calculated.

3.3.5 Presentation of maximal oxygen consumption

Values for $\dot{V}O_{2max}$ were presented as absolute values (L·min^{-1}) and relative to TBM (ml·kg^{-1}·min^{-1}) and FFM (ml·kg$_{FFM}^{-1}$·min^{-1}). All other physiological variables are presented as absolute measures.

Figure 3.1: An example of a male participant with Achondroplasia wearing the online system and facemask.

3.3.6 Statistical analysis

All data were collated onto a personal computer (Macintosh, MacBook Pro) and statistically analysed using SPSS (v22.0, IBM). Data were confirmed parametric following Shapiro-Wilk and Levene's tests. Independent t-tests were used to observe between group differences for absolute and relative measure of $\dot{V}O_{2max}$, HR_{max}, $\dot{V}_E$, V_T, B_f and RER. One tailed Pearson's correlations were performed between percentage of $\dot{V}O_{2max}$, $\dot{V}_{Emax}$ and HR_{max}. Alpha was set at < 0.05 and all results are reported as mean (SD).

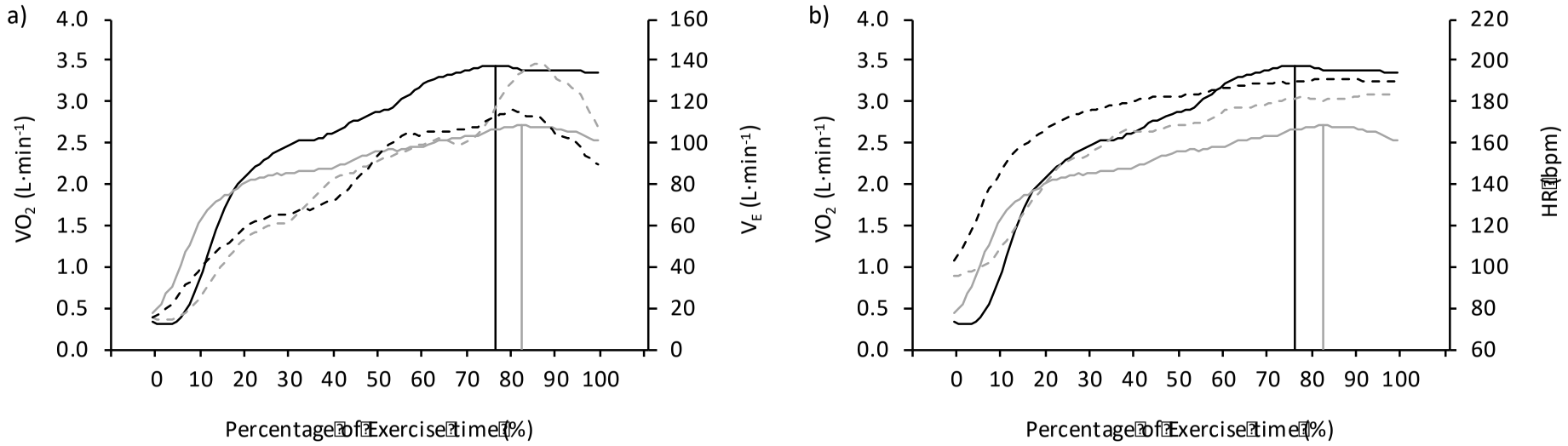

Figure 3.2: a) Absolute $\dot{V}O_2$ (solid lines) and V_E (dotted lines) values plotted over time for one participant with Achondroplasia (grey) and control (black) participant only b) Absolute $\dot{V}O_2$ (solid lines) and HR (dotted lines) values plotted percentage of $\dot{V}O_{2max}$ exercise time for one participant with Achondroplasia (grey) and one control (black) participant only. On both figures, the vertical solid lines represent when $\dot{V}O_{2max}$ was recorded, colours match those described in the legend above. All values are smoothed using a 10-point moving average of raw data.

3.4 Results

Anthropometric measures of all participants are described in Table 2.1 in Chapter 2.

The group with Achondroplasia had a 28.8% lower absolute $\dot{V}O_{2max}$ than controls (P = 0.002, Table 3.1). Relative to TBM and FFM, $\dot{V}O_{2max}$ was not different between groups (P = 0.228 and P = 0.991 respectively, Table 3.1). There was no difference between groups' HR_{max} (P = 0.981), $\dot{V}_E$ (P = 0.079), V_T (P = 0.052), B_f (P = 0.301) or RER attained at $\dot{V}O_{2max}$ (P = 0.662, Table 3.1). There were positive correlations between absolute $\dot{V}O_{2max}$ and TBM and, $\dot{V}O_{2max}$ and FFM for the group with Achondroplasia (r = 0.817, P = 0.004 and r = 0.852, P = 0.002 respectively) and controls (r = 0.423, P = 0.045 and r = 0.626, P = 0.007 respectively, Figure 3.3). Percentage of $\dot{V}O_{2max}$ and $\dot{V}_E$ correlated positively in both groups (Achondroplasia, r = 0.992, P < 0.001; Control: r = 0.995, P < 0.001) as did percentage of $\dot{V}O_{2max}$ and HR (Achondroplasia, r = 0.982, P < 0.001; Control: r = 0.977, P < 0.001, Figure 3.4). No other physiological variable correlated with percentage of $\dot{V}O_{2max}$ in either group.

Table 3.1: Physiological variables collected at $\dot{V}O_{2max}$ for the group with Achondroplasia and controls. Values displayed as mean (SD).

	Achondroplasia		Control
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.63 (0.59)	**	3.44 (0.64)
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	42.2 (5.2)		44.7 (7.7)
$\dot{V}O_{2max}$ ($ml \cdot kg_{FFM}^{-1} \cdot min^{-1}$)	63.2 (8.0)		61.4 (8.7)
HR_{max} (bpm)	193 (13)		193 (11)
$\dot{V}_{Emax}$ ($L \cdot min^{-1}$)	100.8 (20.5)		115.6 (25.7)
V_{Tmax} ($L \cdot min^{-1}$)	1.87 (0.71)		2.30 (0.42)
B_{fmax} ($b \cdot min^{-1}$)	57.0 (9.3)		51.0 (10.2)
RER_{max}	1.40 (0.30)		1.36 (0.18)

HR, heart rate; $\dot{V}_E$, minute ventilation; V_T, tidal volume; B_f, breathing frequency; RER, respiratory exchange ratio; ** $P < 0.001$

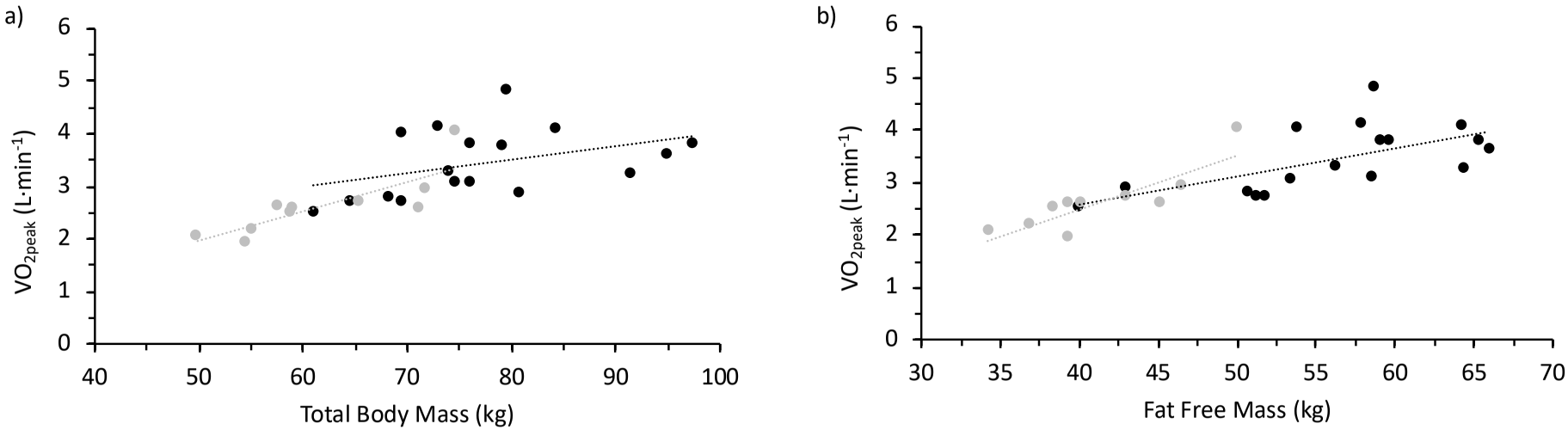

Figure 3.3: Absolute $\dot{V}O_{2max}$ plotted against total body mass for the group with Achondroplasia (grey) and control (black) fitted with a linear trend line (R^2 = 0.667 and 0.179, respectively), and b) mean absolute $\dot{V}O_{2max}$ plotted against fat free mass for the group with Achondroplasia (grey) and control (black) fitted with a linear trend line (R^2 = 0.726 and 0.391, respectively). SD omitted for clarity.

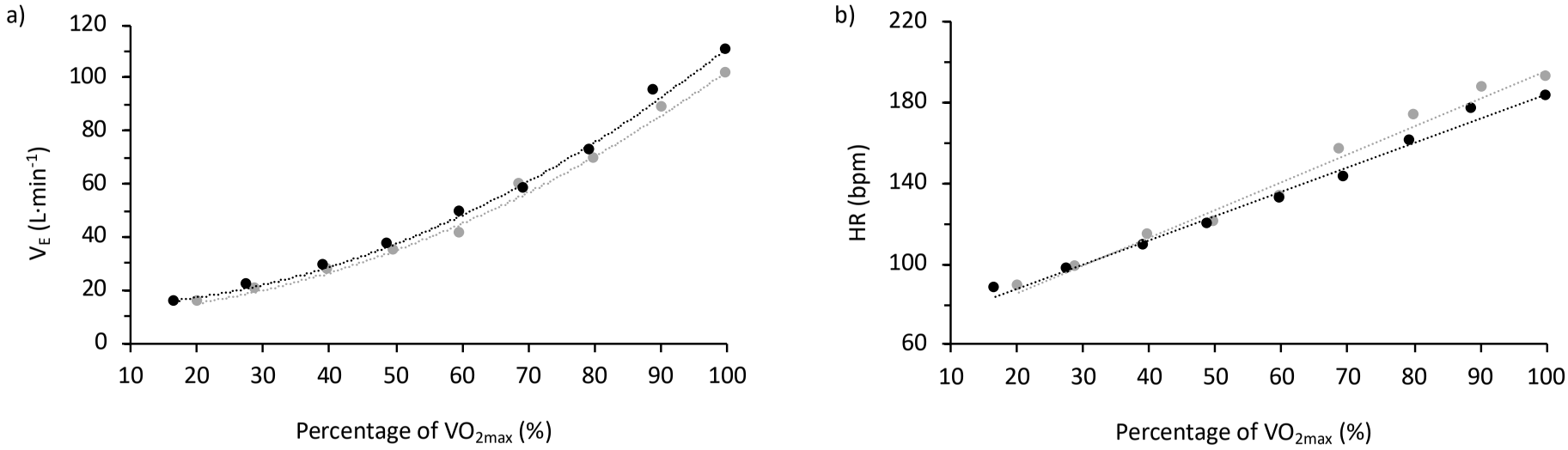

Figure 3.4: a) Minute ventilation (V_E) plotted over percentage of mean absolute $\dot{V}O_{2max}$ for the group with Achondroplasia (grey) and control (black) fitted with a 2nd order polynomial (R^2 = 0.985 and 0.964, respectively), and b) heart rate (HR) plotted over percentage of mean absolute $\dot{V}O_{2max}$ for the group with Achondroplasia (grey) and control (black) fitted with a linear trend line (R^2 = 0.991 and 0.954 respectively). SD omitted for clarity.

3.5 Discussion

The aim of this study was to measure $\dot{V}O_{2max}$ in a group of adults with Achondroplasia and observe any trends between $\dot{V}O_2$ and physiological variables. The hypotheses were met in that the group with Achondroplasia had a lower absolute $\dot{V}O_{2max}$ compared to controls and $\dot{V}O_2$ did correlate positively with HR and $\dot{V}_E$. When $\dot{V}O_{2max}$ was presented relative to TBM or FFM though, no differences were observed between groups.

3.5.1 Maximal oxygen consumption

The main finding from the present study was that absolute $\dot{V}O_{2max}$ was lower in the group with Achondroplasia compared to controls. All respiratory measures ($\dot{V}_E$, V_T and B_f) at $\dot{V}O_{2max}$ were the same between groups. The similarity in respiratory measures is unsurprising as the chest circumference of children with Achondroplasia is the same as controls (Hunter et al., 1996b); this is likely reflected in adults. The lower absolute $\dot{V}O_{2max}$ in the group with Achondroplasia is likely due to a difference in TBM and FFM between groups. This is supported somewhat by the positive correlations between absolute $\dot{V}O_{2max}$ and TBM, and, absolute $\dot{V}O_{2max}$ and FFM for both groups (Figure 3.3). Such correlations are consistent with exercising humans and animals (Rowland, 1989; Weibel and Hoppeler, 2005) and is therefore unsurprising that the difference in groups' absolute $\dot{V}O_{2max}$ is statistically removed when presented relative to TBM and FFM. It is surprising though, that both TBM and FFM are sufficient in removing the observed differences in absolute $\dot{V}O_{2max}$ despite the lower total-body FFM relative to TBM in the group with Achondroplasia

compared to controls (Chapter 2); this is discussed further in section 3.5.2.

Although the present data show $\dot{V}O_{2max}$ relative to TBM is similar between the current groups, this is dissimilar to the findings of Takken et al. (2007). In their study, the absolute $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ relative to TBM of children with Achondroplasia was 44 and 30% less than reference data, respectively. This may be partly due to the fact that Takken et al. used reference data throughout their comparisons rather than collecting their own control data. A more likely reason for the difference in $\dot{V}O_{2max}$ between the adult and child groups with Achondroplasia is the respective description of physical activity. The children with Achondroplasia included in Takken et al. were deemed less physically active than their reference data, whereas the current adults with Achondroplasia self-reported as being physically active to the same level as the control group (i.e. >2 hrs of physical activity per week), Section 2.3 of Chapter 2. Being less physically active moderately correlates with a lower $\dot{V}O_{2max}$ (Lubans et al., 2008; Lubans et al., 2009) and would partly explain the results both within the current Chapter compared to Takken et al. (2007). To further confirm this, measuring $\dot{V}O_{2max}$ in different cohorts with Achondroplasia (i.e. sex, age and activity levels), alongside respective controls groups, as done in the current study, is required.

3.5.2 Relative values of maximal oxygen consumption

In the present group with Achondroplasia, TBM and FFM were sufficient to account for the differences in absolute $\dot{V}O_{2max}$ from controls. This is surprising given that in obese populations of average stature, FFM, not TBM, are more likely to remove the

statistical differences in $\dot{V}O_{2max}$ between obese and non-obese adults (Goran et al., 2000; Dencker et al., 2011). It is possible therefore, that the 7% difference in body fat percentage between the two groups (Chapter 2) was not sufficient to see differences between $\dot{V}O_{2max}$ when relative TBM or FFM.

While FFM is a useful to somewhat normalise $\dot{V}O_{2max}$ to controls', it was assumed in the present Chapter that FFM more accurately represents muscle mass than does TBM. The inclusion, and presentation of, $\dot{V}O_{2max}$ to muscle mass may therefore be more valid and accurate to describe the $\dot{V}O_{2max}$ of individuals with Achondroplasia. Tolfrey et al. (2006) used muscle volume of the thigh to allometrically scale $\dot{V}O_{2max}$ during treadmill running in children; this was due to the exponent of FFM being greater in children compared to adults. There is certainly some weight behind using leg muscle volume to scale $\dot{V}O_{2max}$, however, Tolfrey et al.'s conclusion was based on populations which were of different maturity and development, which influences the amount of muscle mass. Tolfrey et al. also showed that there was a positive correlation between muscle volume and FFM in children but not adults, suggesting that muscle volume is relatively lower in lighter children. In Chapter 2, it was observed that the FFM of the thigh and shank in the group with Achondroplasia were the same as controls when presented relative to total-leg FFM. This would suggest that using muscle volume as an allometric scalar, like that of Tolfrey et al., would be moot for the current groups. For future measurements of $\dot{V}O_{2max}$ or $\dot{V}O_2$ in individuals with Achondroplasia therefore, there is no need to allometrically scale such values. The data presented in this Chapter, and that from Chapter 2, would suggest that $\dot{V}O_{2max}$ or $\dot{V}O_2$ relative to FFM is more appropriate for individuals with

Achondroplasia than when relative to TBM, despite the $\dot{V}O_{2max}$ relative to TBM values being similar to controls'.

3.5.3 Relationships between physiological variables

Lower values of $\dot{V}O_{2max}$ are associated with increased risk of cardiovascular events and health problems (Kodama et al., 2009). With the $\dot{V}O_{2max}$ being similar between the groups in this Chapter, when relative to TBM and FFM, the reported higher mortality of adults with Achondroplasia, due to cardiovascular events, may be due to other factors. This though is beyond the scope of this Chapter and thesis. The group with Achondroplasia in this study, and other populations with Achondroplasia (Horton et al., 1978a; Hecht et al., 1988; Owen et al., 1990; Hoover-Fong et al., 2007), do have higher levels of adiposity, which is linked to an increased risk of cardiovascular events (Després et al., 2006; Després, 2006) and is justification for further work in populations with Achondroplasia.

Cardiovascular related exercise interventions have been carried out in numerous healthy and clinical cohorts to both improve $\dot{V}O_{2max}$ and lower total-body adiposity, which in turn are likely to improve their health and life expectancy (Cuneo et al., 1991; Woodhouse et al., 1999; Helgerud et al., 2007; Carazo-Vargas and Moncada-Jiménez, 2015). The intensities of such programmes are based on relative values of maximal physiological variables, such as $\dot{V}O_{2max}$ or HR_{max}. These relative values are in turn based on either, prior knowledge of the individual's maximal values or, derived from a suitably comparable population's maximal value. With Achondroplasia being disproportionate in mass and stature, the use of maximal values from groups of the

same mass or stature, (e.g. the obese or children) may be misleading for the population. The relationships of $\dot{V}O_2$ and HR, and, $\dot{V}O_2$ and $\dot{V}_E$ were almost identical between groups in this Chapter. This suggests that different types of cardiovascular training aimed at improving either, aerobic capacity or, weight management (e.g. endurance of high intensity) could be based on normative age and sex matched control data (Achten et al., 2002; Milanović et al., 2015). This suggestion however, is based purely on the data from the current Chapter. Further work would certainly be needed to confirm this in other populations with Achondroplasia (e.g. children, females or the elderly). To the author's knowledge, there appears to be no empirical evidence that such interventions (i.e. the use of relative values of control $\dot{V}O_{2max}$ or HR_{max}) have positive effects on the $\dot{V}O_{2max}$ of individuals with Achondroplasia.

3.6 Conclusion

This study aimed to measure $\dot{V}O_{2max}$ and observe correlations between $\dot{V}O_2$ and physiological variable in adults with Achondroplasia and age matched controls. The results showed that absolute $\dot{V}O_{2max}$ of the group with Achondroplasia was significantly lower than controls. When absolute $\dot{V}O_{2max}$ was presented relative to TBM and FFM however, no differences existed between groups. In addition, $\dot{V}O_2$ positively correlated with HR and $\dot{V}_E$ in both groups suggesting that cardiovascular programmes could be implemented in groups with Achondroplasia using the available reference data from controls.

Chapter 4: The oxygen consumption and metabolic cost of walking and running in adults with Achondroplasia

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4.1 Abstract

The disproportionate body mass and leg length of individuals with Achondroplasia may affect their net oxygen consumption ($\dot{V}O_2$) and metabolic cost (C) when walking and running compared to those of average stature (controls). The aim of this study was to measure submaximal $\dot{V}O_2$ and C during a range of set walking speeds (SWS; 0.56 - 1.94 $m \cdot s^{-1}$, increment 0.28 $m \cdot s^{-1}$), set running speeds (SRS; 1.67 - 3.33 $m \cdot s^{-1}$, increment 0.28 $m \cdot s^{-1}$) and a self-selected walking speed (SSW). $\dot{V}O_2$ and C was presented relative to total-body mass ($_{TBM}$) and fat free mass ($_{FFM}$) while gait speed was normalised to leg length using Froude's number (*Fr*). The $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ of the group with Achondroplasia were on average 29 and 35% greater during SWS ($P < 0.05$) and 12 and 18% higher during SRS ($P < 0.05$) than controls, respectively. The C_{TBM} and C_{FFM} of the group with Achondroplasia were 29 and 33% greater during SWS ($P < 0.05$) and 12 and 18% greater during SRS ($P < 0.05$) than controls, respectively. There was no difference in SSW $\dot{V}O_{2TBM}$ or $\dot{V}O_{2FFM}$ between groups ($P > 0.05$), but C_{TBM} and C_{FFM} at SSW were 23 and 29% higher ($P < 0.05$) in the group with Achondroplasia compared to controls, respectively. $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ correlated with Fr for both groups ($r = 0.984 - 0.999$, $P < 0.05$). Leg length accounted for the majority of the higher $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ in the group with Achondroplasia, but further work is required to explain the higher C_{TBM} and C_{FFM} at all speeds in the group with Achondroplasia compared to controls.

Key words: Achondroplasia; Oxygen Consumption; Metabolic Cost; Walking; Running

4.2 Introduction

The shorter lower limbs of populations with Achondroplasia compared to controls (Chapter 2) is likely to affect functional tasks, such as walking, which in turn is likely to affect the oxygen consumption ($\dot{V}O_2$) and metabolic cost of locomotion (C). For example, a higher stride frequency is observed in proportionally shorter statured groups compared to taller individuals during walking and running at the same speeds (Minetti et al., 1994). This in turn leads to a higher $\dot{V}O_2$ in the shorter groups at set walking speeds (Rowland and Green, 1988a). When gait speed is increased, such as during incremental walking and running, a positive curvilinear trend of absolute $\dot{V}O_2$ exists in numerous cohorts (Rowland and Green, 1988a; Minetti et al., 1994; Schepens et al., 2004; van den Hecke et al., 2007). In proportionally shorter statured groups there is a higher $\dot{V}O_2$ compared to taller groups when walking and running at set speeds (Rowland and Green, 1988a; Minetti et al., 1994; Schepens et al., 2004; Ludlow and Weyand, 2015). As observed in Chapter 3, the differences in maximal $\dot{V}O_2$ between the groups was accounted for by total-body mass (TBM) and fat free mass (FFM), and have also been observed during incremental exercise in the obese (Goran et al., 2000; Browning et al., 2006).

Accounting for leg length when presenting horizontal speed, as done with Froude's number (*Fr*), further removes the observed difference in $\dot{V}O_2$ between shorter and taller groups during incremental or steady state exercise (Ferretti et al., 1991; Minetti et al., 1994; Steudel and Beattie, 1995; Steudel-Numbers et al., 2007; P. A. Kramer and Sylvester, 2012). The size, and therefore mass, of the torso between individuals with Achondroplasia and controls is similar, but with groups' the leg lengths being

different, the ratio of torso-to-leg mass is greater in individuals with Achondroplasia (Chapter 2); this ratio has been shown to lead to a higher $\dot{V}O_2$ and C during walking and running in controls (Griffin et al., 2003; Browning et al., 2006; Beekley et al., 2007; Peyrot et al., 2009; McCormick, 2014). The shorter legs of individuals with Achondroplasia is likely contribute to a higher $\dot{V}O_2$ than controls when exercising at the same speed (Minetti et al., 1994). Therefore, scaling $\dot{V}O_2$ separately to either leg length or body mass during incremental exercise would likely under- and over-predict the $\dot{V}O_2$ of individuals with Achondroplasia compared to controls. To the author's knowledge though, there are no data pertaining to the measurement of $\dot{V}O_2$ in individuals with Achondroplasia during incremental exercise, let alone the scaling of $\dot{V}O_2$during incremental exercise.

$\dot{V}O_2$ is useful to describe the cardiovascular response during exercise, but C describes the oxygen demand over a given distance (P. A Kramer and Sarton-Miller, 2008). For the same incremental walking that exhibits a positive trend of $\dot{V}O_2$ described above, a U-shaped curve of C exists with clear local minima (Ferretti et al., 1991; Minetti et al., 1994; McCann and Adams, 2002a; P. A Kramer and Sarton-Miller, 2008). This minimum suggests that respective slower and faster walking speeds are less economic, or, have a higher C (i.e. greater $\dot{V}O_2$ is required for the given distance). A local minima is observed at different speeds when stride frequency is manipulated, suggesting an optimal stride frequency for different speeds (Minetti et al., 1995). The local minima of C during walking is observed around self-selected walking (SSW) speed (Ralston, 1958), but has not been measured alongside set walking speeds in adults groups of shorter stature (Ferretti et al., 1991; Minetti et al., 1994; Minetti et

al., 2002). In groups of shorter stature, their higher C can be accounted for by their higher stride frequency (Minetti et al., 1994). The inclusion of *Fr* within the scaling procedures of C can help the normalise stride frequency of set gait speeds while SSW may explain some of the expected U-shape curve of walking C in both individuals with Achondroplasia and controls.

Therefore, the overriding aim of this study was to observe the relationship between $\dot{V}O_2$ and incremental walking and running in adults with Achondroplasia. The primary objectives were to:

1) collect submaximal $\dot{V}O_2$ in adults with Achondroplasia and controls at differing absolute and relative (SSW) intensities of walking and running;
2) convert $\dot{V}O_2$ into C values in all participants and;
3) attempt to account for any differences in $\dot{V}O_2$ and C by normalising to body masses and leg length.

It was hypothesised that the group with Achondroplasia would have a higher $\dot{V}O_2$ and C at all walking and running speeds, but statistical differences would lessen once presented relative to TBM and FFM and by including leg length as a speed scaler.

4.3 Methods

4.3.1 Participants

Ten adults with Achondroplasia and 17 age matched controls that were free from lower limb injury volunteered to participate in the study and are described in Table 2.1 in Chapter 2.

4.3.2 Anthropometric measures

Leg length (m) of all participants was measured as the distance from the anterior iliac spine to the medial malleolus of the ankle while standing. Participants' TBM (kg) was obtained using electronic scales (SECA 813, CA 91710 Chino, USA) while barefooted and wearing minimal clothing. FFM was obtained using Dual energy x-ray absorptiometry (DEXA), described in detail in section 2.3.2 of Chapter 2.

4.3.3 Speed of locomotion

SSW trials were completed by participants conforming to a habitual walking pace around the laboratory (~40 m). Each participant passed through two timing gates (1 m apart), three times. SSW speeds ($m \cdot s^{-1}$) were calculated and recorded as an average of the three trials and used in $\dot{V}O_2$ assessment where individuals' SSW intersected absolute speeds described below. $\dot{V}O_2$ collection apparatus (described in the next section) was worn throughout all exercise trials which were conducted on a motorised treadmill (Woodway PPS70, LOCAtion). Treadmill speeds were set at 0.56 - 1.94 $m \cdot s^{-1}$ (increment 0.28 $m \cdot s^{-1}$) for walking and 1.67 - 3.33 $m \cdot s^{-1}$ (increment 0.28

$m \cdot s^{-1}$) for running, as described in Ferretti et al. (1991) and Minetti et al. (1994; 2002). All trials were completed at 1% gradient to replicate outdoor conditions (Jones and Doust, 1996) with each stage being 4 minutes in duration to attain steady state, again replicating Ferretti et al. (1991) and Minetti et al. (1994; 2002). Participants rested for ~5 minutes following all walking trials to reduce $\dot{V}O_2$. Where participants could not maintain running speed for the entirety of the 4 minutes during any walking and running intensity, the stage was omitted from analysis and the testing protocol terminated.

4.3.4 Oxygen uptake, metabolic cost and their presentation

Expired gases were collected and analysed using portable breath-by-breath indirect calorimetry (Metamax 3B, Cortex, Leipzig Germany), which was calibrated to the manufacturer's guidelines prior to testing. The portable indirect calorimeter (weight = 1 kg) and a fitted face mask (Hans Rudolph V2, dead space between 125 – 143 ml) were worn by participants during the exercise bout. Prior to exercise testing, participants lay supine for 5 minutes so that resting metabolic rate ($L \cdot min^{-1}$) could be measured. Gross $\dot{V}O_2$ for each intensity was recorded with net $\dot{V}O_2$ calculated by subtracting resting metabolic rate from gross $\dot{V}O_2$ as conducted in Minetti et al. (1994; 2002), hereafter net $\dot{V}O_2$ is referred to as '$\dot{V}O_2$'. Steady state $\dot{V}O_2$ was determined by a respiratory exchange ratio < 1.0 and by a visual plateau of $\dot{V}O_2$ over the final minute of exercise, with $\dot{V}O_2$ recorded as a rolling average of 6 measurements (every 10 s) for each exercise intensity. C was presented as the amount of $\dot{V}O_2$ required to complete 1 km at each gait speed, given as ($L \cdot km^{-1}$). $\dot{V}O_2$

and C were then normalised to TBM ($\dot{V}O_{2TBM}$) and C_{TBM}, respectively) and FFM ($\dot{V}O_{2FFM}$ and C_{FFM}, respectively). All $\dot{V}O_2$ and C values were presented against absolute walking and running speeds, and against dimensionless *Fr*, given as: velocity^2 (m·s^{-1}) ÷ $\sqrt{\text{leg length (m)} \cdot 9.81\ (\text{m}\cdot\text{s}^{-2})}$.

4.3.5 Statistical analysis

All data were collated onto a personal computer (Macintosh, MacBook Pro) and analysed using SPSS (v22.0, IBM). Data were assumed parametric following Shapiro-Wilk and Levene's tests. To avoid a Type I error in the comparisons between groups' $\dot{V}O_2$ and C measures, a 7x2 mixed design ANOVA was used to identify significant effects. However, only the differences between groups were of interest. Due to the subtle differences in leg length between the group with Achondroplasia and control participants, respectively, interpretation of the interaction between *Fr* and $\dot{V}O_2$ and C is more difficult. Due to the linear relationship between $\dot{V}O_2$ and *Fr* presented elsewhere (P. A Kramer and Sarton-Miller, 2008), the mean of each groups' *Fr* and $\dot{V}O_2$ recorded at each walking and running condition using a Pearson's correlation. Due to the curvilinear relationship between C and speed, the relationship between *Fr* and C was not inferentially compared. All results are reported as means (SD).

4.4 Results

4.4.1 Participant anthropometrics

The description of participants' anthropometrics are given in Table 2.1 of Chapter 2.

4.4.2 Self-selected walking

The group with Achondroplasia were 23% slower than controls at SSW (Achondroplasia, 1.02 (0.13) $m \cdot s^{-1}$; control 1.33 (0.14) $m \cdot s^{-1}$, $P < 0.001$).

4.4.3 Incremental exercise

During $\dot{V}O_2$ assessment, both groups completed all walking speeds. However, only 50% of the group with Achondroplasia managed to obtain steady state running at 2.50 $m \cdot s^{-1}$ and only 20% maintained steady state running at 2.78 $m \cdot s^{-1}$. Therefore, $\dot{V}O_2$ and C values collected at all walking speeds and at running speeds 1.67 – 2.22 $m \cdot s^{-1}$ were inferentially analysed (Figures 4.1 and 4.2).

4.4.4 Oxygen consumption

$\dot{V}O_{2WBM}$ were on average 29% greater in the group with Achondroplasia at all absolute walking speeds apart from SSW where no difference was observed (Figure 4.1a). Similarly, the group with Achondroplasia had an average 35% greater $\dot{V}O_{2FFM}$ at all absolute walking speeds compared to the control group, with no difference being found between groups at SSW (Figure 4.1b). There was no difference in $\dot{V}O_{2TBM}$ between groups at running speed 1.67 $m \cdot s^{-1}$, but the group with Achondroplasia had

a 14% and 12% higher $\dot{V}O_{2TBM}$ than controls at running speeds 1.94 and 2.22 $m \cdot s^{-1}$ (Figure 4.1a). A higher $\dot{V}O_{2FFM}$ was observed in the group with Achondroplasia compared to controls for all running speeds (Figure 4.1b).

4.4.5 Metabolic Cost

On average, the group with Achondroplasia had a 29% higher walking C_{TBM} and 33% higher walking C_{FFM} when compared to controls (Figure 4.1c and 4.1d). Running C_{TBM} were the same between groups at 1.67 and 2.22 $m \cdot s^{-1}$ ($P > 0.05$) whereas the group with Achondroplasia had a higher C_{TBM} than controls at 1.94 $m \cdot s^{-1}$ ($P < 0.05$, Figure 4.1c). The C_{FFM} during running was, on average, 18% higher at all running speeds in the group with Achondroplasia compared to controls ($P < 0.05$, Figure 1d).

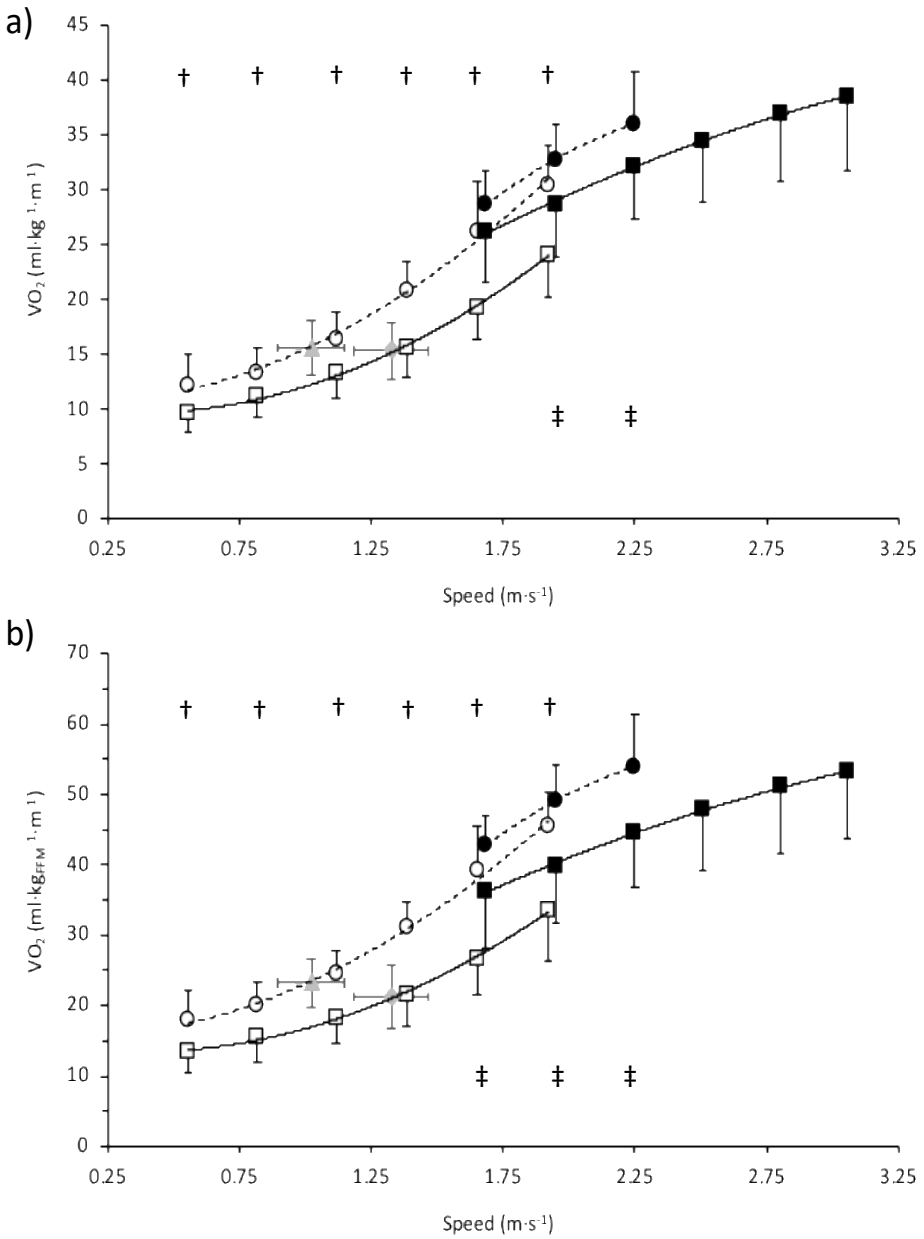

*Figure 4.1a and b: Mean SD (error bars) of net oxygen consumption ($\dot{V}O_2$) for the group with Achondroplasia (○) and control (□) when walking (open) and running (closed) at absolute and SSW speeds ($m \cdot s^{-1}$); SSW are presented for the group with Achondroplasia (▲) and control (◆) respectively. $\dot{V}O_2$ is presented relative to a) total-body mass and b) relative to fat free mass * P < 0.05 at SSW between groups; † P < 0.05 between groups at paired walking speeds; ‡ P < 0.05 between groups at paired running speeds. $\dot{V}O_2$ and speed relationships are fitted with a 2nd order polynomial, with the broken and continuous lines being the trends for the* group with Achondroplasia *and controls, respectively.*

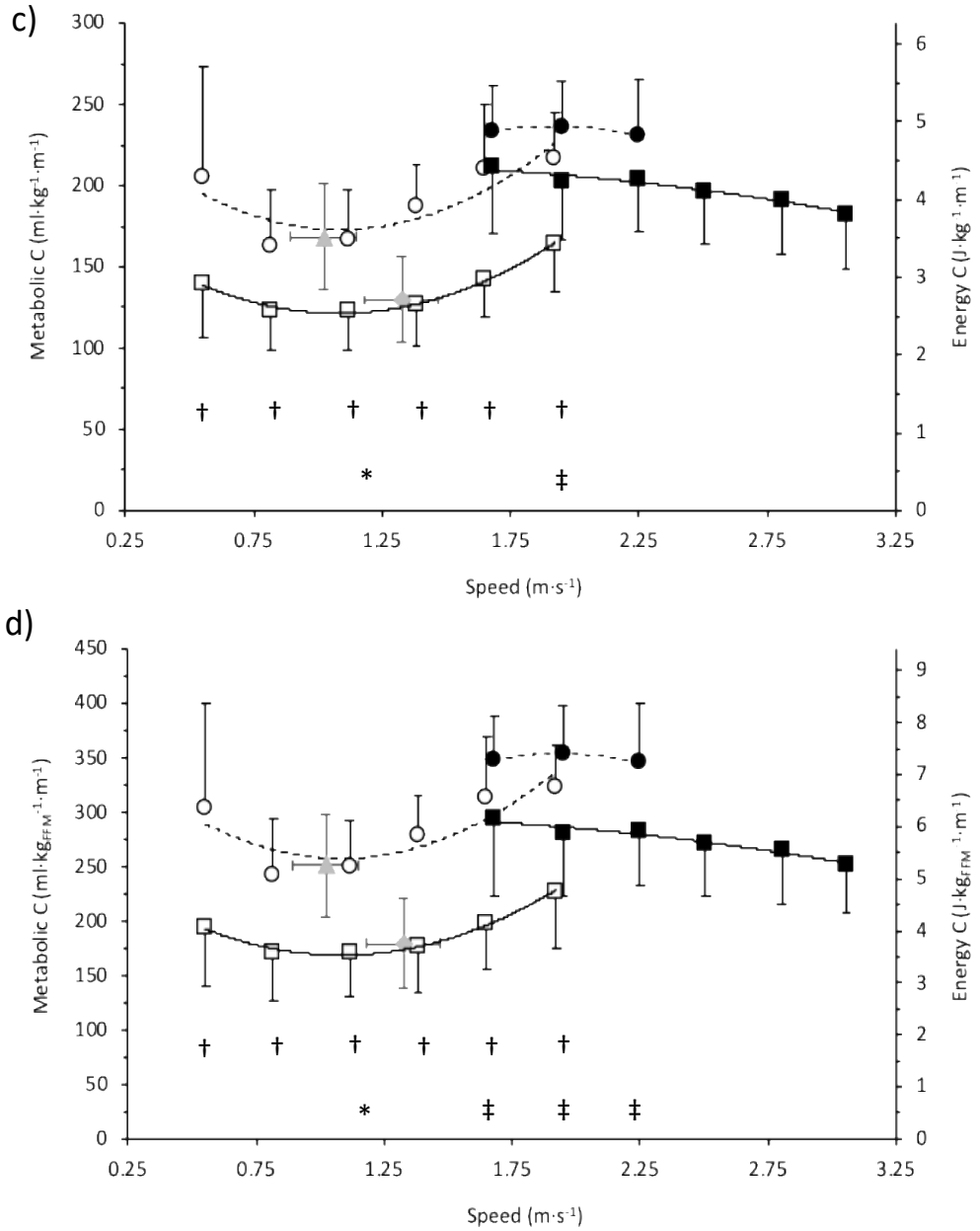

*Figure 4.1c and d: Mean SD (error bars) of net and metabolic cost (C) for the group with Achondroplasia (○) and control (□) when walking (open) and running (closed) at absolute and SSW speeds (m·s^{-1}); SSW are presented for the group with Achondroplasia (▲) and control (◆) respectively. C is presented relative to c) total-body mass and d) relative to fat free mass; C is also presented as the energy cost by considering 1 ml of O_2 = 20.9 J. * P < 0.05 at SSW between groups; † P < 0.05 between groups at paired walking speeds; ‡ P < 0.05 between groups at paired running speeds. C and speed relationships are fitted with a 2nd order polynomial, with the broken and continuous lines being the trends for the* group with Achondroplasia *and controls, respectively.*

4.4.6 Froude comparisons

Strong positive correlations existed between *Fr* and $\dot{V}O_{2TBM}$ when walking (r = 0.998, P = 0.001) and running (r = 0.994, P = 0.070) for the group with Achondroplasia, and also for controls when walking (r = 0.997, P < 0.001) and running (r = 0.985, P < 0.001, Figure 4.2a, Table 4.1). There were also strong positive correlations between *Fr* and $\dot{V}O_{2FFM}$ when walking (r = 0.999, P < 0.001) and running (r = 0.993, P = 0.036) for the group with Achondroplasia and for the control group when walking (r = 0.997, P < 0.001) and running (r = 0.984, P < 0.001, Figure 4.2b, Table 4.1).

Table 4.1: R^2 values for the relationships between Froude's number and relative presentations of oxygen consumption ($\dot{V}O_2$) in individuals with Achondroplasia and controls during walking and running.

	Achondroplasia		Control	
	Walking	Running	Walking	Running
$\dot{V}O_{2TBM}$	0.997	0.988	0.994	0.985
$\dot{V}O_{2FFM}$	0.997	0.987	0.994	0.984

$\dot{V}O_{2TBM}$, oxygen consumption relative to total-body mass; $\dot{V}O_{2FFM}$, oxygen consumption relative to fat free mass. All correlations significant to P < 0.001.

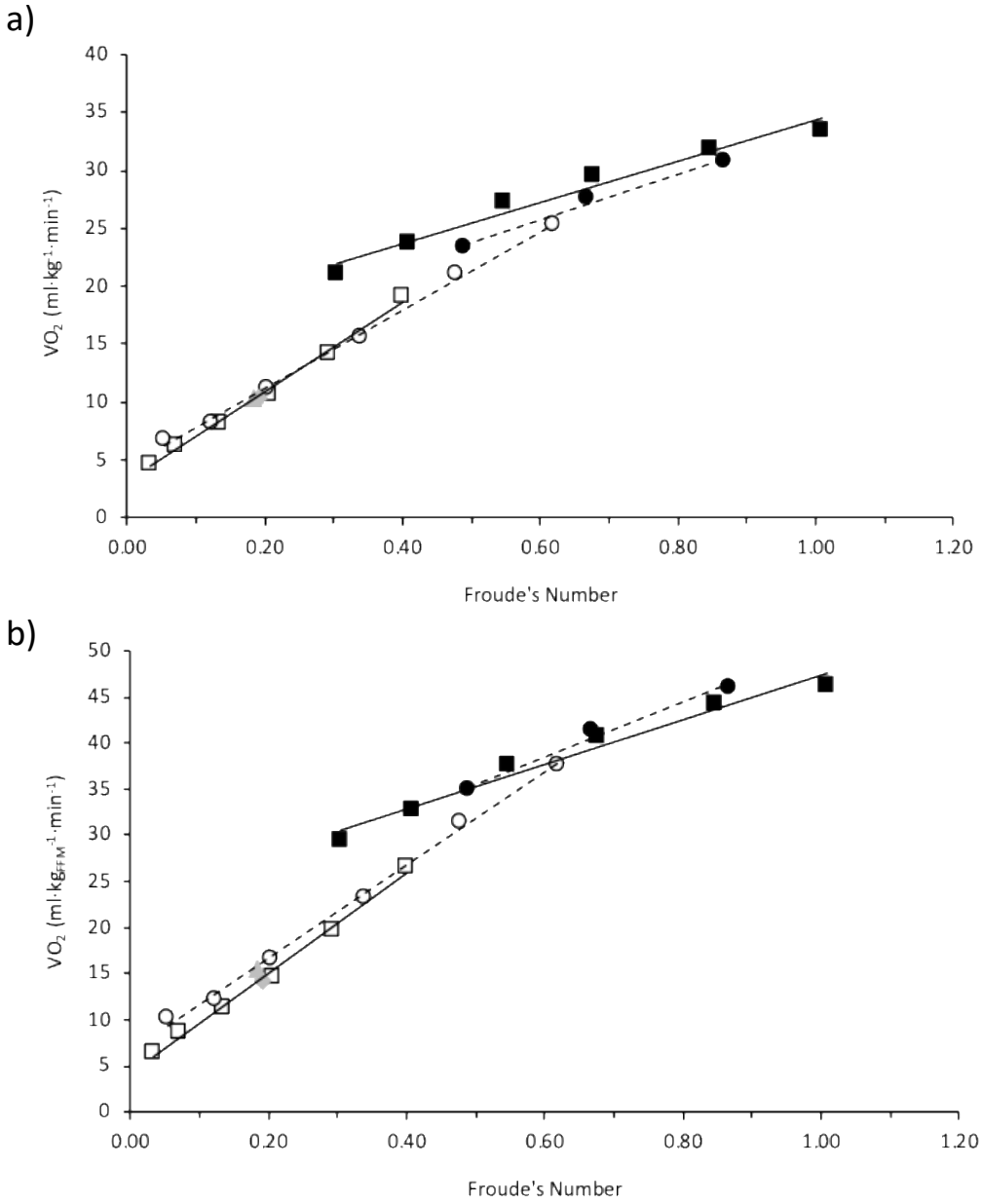

Figure 4.2a and b: Mean net oxygen consumption ($\dot{V}O_2$) for the group with Achondroplasia (○) and control (□) when walking (open) and running (closed) at mean Froude's numbers (Fr); SSW are presented for the group with Achondroplasia (▲) and control (◆) respectively. $\dot{V}O_2$ is presented relative to a) total-body mass and b) relative to fat free mass; C is also presented as the energy cost by considering 1 ml of O_2 = 20.9 J. $\dot{V}O_2$ and Fr relationships are fitted with a linear trend line with the broken and continuous line being the trends for the group with Achondroplasia and control group, respectively. SD is omitted for clarity.

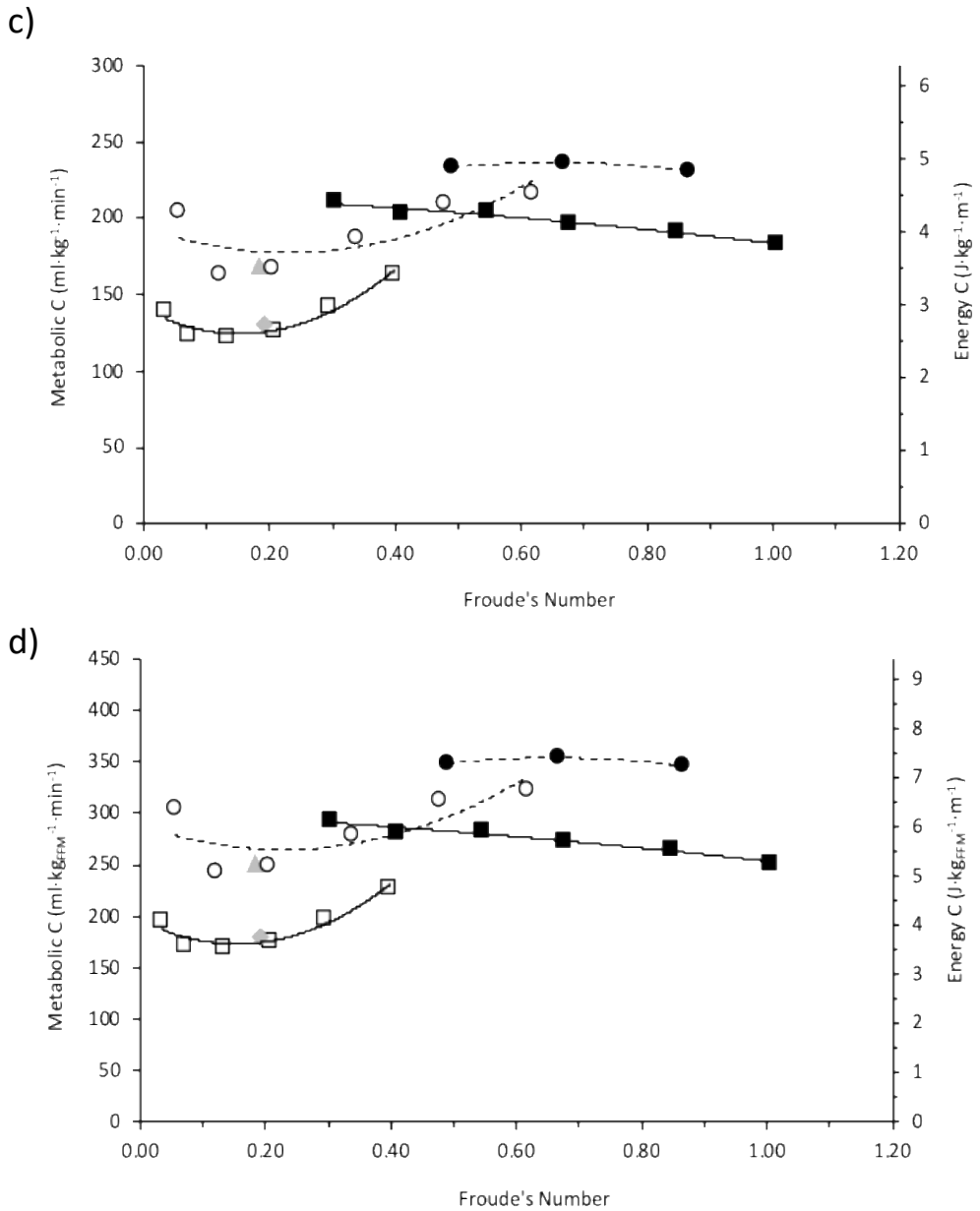

Figure 4.2c and d: Mean net metabolic cost (C) for the group with Achondroplasia (○) and control (□) when walking (open) and running (closed) at mean Froude's numbers (Fr); SSW are presented for the group with Achondroplasia (▲) and control (◆) respectively. C is presented c) relative to total-body mass and d) relative to fat free mass; C is also presented as the energy cost by considering 1 ml of O_2 = 20.9 J. C and Fr relationships are fitted with a 2nd order polynomial with the broken and continuous line being the trends for the group with Achondroplasia and control group, respectively. SD is omitted for clarity.

4.5 Discussion

The main aim of this study was to attain $\dot{V}O_2$ and C profiles during incremental walking and running in adult males with Achondroplasia and compare the results to controls. Further to this, in an attempt to account for potential differences, $\dot{V}O_2$ and C were presented relative to body masses and leg length in both groups. The hypotheses were partially met in that: 1) the group with Achondroplasia had a greater $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ during walking and running compared to controls and had a higher C_{TBM} and C_{FFM} (i.e. a greater $\dot{V}O_2$ for a given distance) at all walking speeds compared to controls; and, 2) leg length explained some of the difference in $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ between groups but not for the comparison of C_{TBM} and C_{FFM}.

4.5.1 Oxygen consumption

Other than SSW, the $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ were higher at every walking and running speed in the group with Achondroplasia compared to controls. This is similar to previous reports, where shorter statured groups have higher $\dot{V}O_{2TBM}$ than taller counterparts during locomotion (Rowland and Green, 1988a; Minetti et al., 1994; Schepens et al., 2004; Ludlow and Weyand, 2015). The higher $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ at set speeds in the group with Achondroplasia is most likely due to them having a higher stride frequency than controls. Minetti et al. (1994) partially confirmed that higher stride frequency was the cause of higher $\dot{V}O_{2TBM}$ in their African Pygmy group. While stride frequency was not measured in the present Chapter, the data presented by Minetti et al. (1994) would infer some similarities between groups. The Pygmies included in Minetti et al. (1994) were 16 cm taller than the group with

Achondroplasia presented here, while the two control groups were 1 cm different. With Pygmies being smaller and having a higher stride frequency compared to controls, it is likely that the even smaller group of individuals with Achondroplasia had a similar, if not higher, stride frequency to the Pygmies.

While this Chapter does not directly measure factors that explain the higher $\dot{V}O_2$ of the group with Achondroplasia for a given speed of locomotion, there are a number of mechanisms that could explain these data. Firstly, the probable higher stride frequency of the group would lead to a greater amount of internal mechanical work being done compared to controls, as observed in other shorter statured groups (Ferretti et al., 1991; Minetti et al., 1994; DeJaeger et al., 2001; Minetti et al., 2002; Schepens et al., 2004; Weyand et al., 2010). A higher internal work not only requires energy to complete the work because it elicits a greater rate of muscular contraction. Assuming the fibre type distribution is the same between groups, the rate of muscular contraction is likely to alter the force-velocity relationship of the muscles within the individuals with Achondroplasia (Fletcher and MacIntosh, 2017). In this scenario, the muscles of the group with Achondroplasia would be producing less force due to the quicker movement of the limbs. Muscle activation could therefore be higher in the group with Achondroplasia to recruit the fibres required to maintain locomotion forces, therefore leading to a higher oxygen demand (Mian et al., 2006). However, internal work, force production (relative to gait requirements) and muscle activation have not been measured in individuals with Achondroplasia during gait; therefore, more work is required in this area to help confirm these theories. To try to account for the assumed higher stride frequency of individuals with

Achondroplasia, and therefore eliminate some of the above, horizontal speed was presented as a normalised value by incorporating leg length in the form of *Fr*.

4.5.2 Comparisons of Froude's number

In the current study, *Fr* could explain the variability of the groups' $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ by as much as 98.7% (Figure 4.2a and 4.2b, Table 4.1). This matches much of the literature where *Fr* has been used to compare the relationship between leg length and $\dot{V}O_2$ during locomotion. Ferretti et al. (1991) and Minetti et al. (1994) showed African Pygmy's $\dot{V}O_{2TBM}$ and *Fr* trends are similar to controls, with Minetti et al. (2002) also observing similar findings in patients with GHD. The similarity of the $\dot{V}O_2$ and *Fr* trends between groups suggests that the difference in groups' $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ can be accounted for by the shorter legs of individuals with Achondroplasia. The similarity in the slopes are not surprising given that $\dot{V}O_2$ correlates well with TBM and FFM (Goran et al., 2000; Weibel and Hoppeler, 2005). It is important to note here though, that although the relationships appear similar between the groups and between relative values of $\dot{V}O_2$ (Table 4.1), each mass relationship should not be used to estimate another. For example, in both groups a *Fr* of ~0.30 elicits a $\dot{V}O_{2TBM}$ ~15 $ml \cdot kg^{-1} \cdot min^{-1}$, whereas the same *Fr* elicits a $\dot{V}O_{2FFM}$ ~20 $ml \cdot kg^{-1} \cdot min^{-1}$. While the inclusion of *Fr* accounted for the difference in $\dot{V}O_2$ by providing similar slopes for both $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ between groups respectively, *Fr* did not explain the difference in C between groups.

4.5.3 Metabolic cost

While $\dot{V}O_2$ is a useful parameter, it only describes the rate at which O_2 is used, not the usage per unit distance. In contrast, C provides O_2 usage per unit distance and can be converted into energy cost, assuming an energy equivalent of 20.9 J per 1 ml O_2 (Figure 4.1b and 4.1d) and describes the energetic cost per unit mass and distance. Despite accounting for mass and distance travelled, the group with Achondroplasia exhibited a higher C_{TBM} and C_{FFM} during walking and running than controls. In addition, C_{TBM} and C_{FFM} at SSW occurred near the local minima of the trend lines for both groups but remained different between groups. The factors that may account for the difference in C between groups are again not investigated directly in this Chapter, but the available literature does allow insight into potential reasons to why these differences exists.

The torso size (top of head to base of the pelvis, Chapter 2 Table 2.3) of individuals with Achondroplasia and controls is the same, but their legs are shorter. Individual with Achondroplasia therefore have a greater upper body (torso, as defined above, and both arms)-to-leg mass ratio than controls. Where additional mass is added to the torso of individuals exercising on a treadmill, a higher C is observed compared to individuals without additional mass (Griffin et al., 2003; Browning et al., 2006; Beekley et al., 2007; Browning et al., 2007; Peyrot et al., 2009; McCormick, 2014); the converse is observed when body weight is reduced through assisted treadmill running (Grabowski and Kram, 2008). While the present group with Achondroplasia were lighter than the control group, the additional torso mass of the group would have implications for the contractile properties of their smaller lower leg muscles

during stance. Firstly, relative to lower leg muscle mass, a greater vertical ground reaction force (vGRF) could be observed in the group with Achondroplasia during locomotion. The probable smaller muscle fibres of the lower limbs within individuals with Achondroplasia would therefore do more external work during the braking phase of stance to compensate for the relatively larger vGRF, eliciting a higher C (Pontzer, 2005; Grabowski and Kram, 2008). It is also possible that during the propulsion phase of gait, there is greater coactivation of the hamstrings in individuals with Achondroplasia, due to a greater joint laxity (reported through functional assessments (Bober et al., 2008)). Previously, a higher coactivation during gait contribute to negative work which has been associated with a higher C (Mian et al., 2006).

These theories to why the group with Achondroplasia have a persistently higher C during gait (e.g. mechanical work, force production and tendon compliance), are not substantiated in the literature though. Further work is therefore required to help explain the difference in C between individuals with Achondroplasia and controls.

4.6 Conclusion

This Chapter aimed to observe the relationship between $\dot{V}O_2$ and incremental walking and running speeds in adult males with Achondroplasia. To the author's knowledge, this is the first study to compare $\dot{V}O_2$ and C during walking and some running speeds in adults with Achondroplasia and controls. The main findings are that 1) the $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ are higher in individuals with Achondroplasia at all

walking and running speeds compared to controls; 2) $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ is not different between groups at SSW; 3) C_{TBM} and C_{FFM} are higher in individuals with Achondroplasia than controls at all walking and running speeds, and 4) the inclusion of leg length as a speed scaler helped explain $\dot{V}O_2$ differences but not C differences between groups. The higher $\dot{V}O_{2TBM}$ and $\dot{V}O_{2FFM}$ in individuals with Achondroplasia are most likely due to a higher stride frequency, while their higher C_{TBM} and C_{FFM} is likely due to anthropometrical differences compared to controls.

Chapter 5: The *in vivo* specific force of the vastus lateralis in adults with Achondroplasia

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5.1 Abstract

Force production in able-bodied individuals (controls) is proportional to muscle size. Given the disproportionate nature of individuals with Achondroplasia, normalising to anatomical cross-sectional area (ACSA) is inappropriate. The aim of this study was to assess specific force of the vastus lateralis (VL) in 10 adults with Achondroplasia (22 ±3 yrs) and 17 age matched controls (22 ±2 yrs). Isometric torque (iMVCτ) of the dominant knee extensors (KE) and *in vivo* measures of VL muscle architecture, volume, activation and patella tendon moment arm were used to calculate VL physiological CSA (PCSA), fascicle force and specific force in both groups. Muscle volume was 53% smaller in the group with Achondroplasia than controls ($P < 0.001$). KE iMVCτ was 63% lower in the group with Achondroplasia compared to controls ($P < 0.001$). Activation and moment arm length were similar between groups ($P > 0.05$), but coactivation of the bicep femoris was 70% more in the group with Achondroplasia than controls ($P < 0.001$). The group with Achondroplasia had 58% less PCSA ($P < 0.001$), 29% lower fascicle force ($P < 0.001$) and 29% lower specific force than controls ($P = 0.012$). The smaller VL specific force in individuals with Achondroplasia may be attributed to infiltration of fat and connective tissue, rather than to any difference in myofilament function.

Key Words: Achondroplasia; Specific Force, Vastus Lateralis

5.2 Introduction

It was shown in Chapter 3 that differences in absolute maximal oxygen consumption ($\dot{V}O_{2max}$) between an adults with Achondroplasia and age matched average statured population (controls) is not different when relative to total-body mass and fat free mass. These data suggested that the aerobic capacity of muscle between groups was similar. Chapter 4 however, showed that submaximal oxygen consumption ($\dot{V}O_2$) and metabolic cost (C) was higher in adults with Achondroplasia compared to controls when walking and running at set speeds. Given than $\dot{V}O_2$ was similar between groups when leg length was accounted for, the higher C in the group with Achondroplasia is likely due to biomechanical differences, such as force production and activation profiles of gait related muscles. Both of which are currently unreported in populations with Achondroplasia.

The contribution of force from the muscle in proportionally smaller groups has been investigated with force production appearing to be proportional to muscle morphology, such as muscle volume and fascicle length (Y. J. Janssen et al., 1999; Morse et al., 2008). Individuals with Achondroplasia have less fat free mass of the thigh compared to controls (Chapter 2) and therefore likely produce less force from the thigh muscles; this is obserevd in children with Achondroplsia during knee exenstion (Takken et al., 2007). The group with Achondroplasia having a similar amount of fat free mass of the thigh relative to total-limb fat free mass though (Chapter 2), it is probable that there is a similar relative force production of the thigh muscles compared to controls. Although there are strength measures in children with Achondroplasia (Takken et al., 2007), there is no comparison of force production

capacity in adults with Achondroplasia to controls, nor is there any measure of muscle morphology or size to present relative strength values.

Muscle morphology, defined here as muscle size and architecture, is a primary determinant of muscle function and can account for some of the differences observed in proportionally smaller people (Kanehisa et al., 1994; Sartorio and Narici, 1994; Bottinelli et al., 1997; Y. J. Janssen et al., 1999; Morse et al., 2008; O'Brien et al., 2010c; O'Brien et al., 2010d). Primarily, the determinants of muscle force are: muscle shortening velocity, physiological cross sectional area (PCSA) of the muscle, fascicle length and muscle volume, respectively (Narici et al., 1992). Neural factors of the agonists and antagonists also contribute to force production as well as the biomechanical form of the joint (Merton, 1954; Maganaris et al., 1998; Maganaris, 2001). In numerous clinical conditions, such as the aging or individuals with Cerebral Palsy, the prevalence of weakness corresponds with functional impairments such as slower walking speeds and reduced performance of functional tasks (Hurley et al., 1998; Dodd et al., 2002).

The measurement of specific force integrates the measurement of muscle size, architecture, neural capacity and moment arm, providing a normalised value of force production (Erskine et al., 2009; Stebbings et al., 2014). While there is some variability in specific force, the values are similar across different cohorts, muscles and species (Degens et al., 1995; Maganaris et al., 2001; Morse et al., 2008; Erskine et al., 2009; Stebbings et al., 2014). While specific force is similar between muscle groups, such measurements in muscles of the leg, e.g. the vastus lateralis (VL), allow

an indication of gait ability and $\dot{V}O_2$. The measurement of specific force, which accounts for neuromuscular, biomechanical and architectural properties of the myotendinous unit, should therefore allow for an accurate assessment and comparison of relative force production between individuals with Achondroplasia and controls.

The aim of this study therefore is to assess specific force in adult males with Achondroplasia.

The objectives of this study were to:

1) measure the maximal voluntary contraction of the knee extensors in adults with Achondroplasia and compare to controls;
2) identify the neural, morphological and biomechanical determinants of the vastus lateralis in adults with Achondroplasia and compare to controls;
3) account for any difference in torque production between groups by calculating specific force.

5.3 Methods

5.3.1 Participants

Ten adults with Achondroplasia and 17 age matched controls that were free from lower limb injury volunteered to participate in the study and are described in Table 2.1 in Chapter 2.

5.3.2 Specific force calculation

5.3.2.1 Strength measurements

The torque derived from isometric maximal voluntary contraction (iMVCτ) of the dominant KE (Achondroplasia n = 9/10 right leg, control n = 16/17 right leg) were recorded using an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA). Participants were seated upright with the dynamometer and chair positioned in accordance with the calibration guidelines given by the manufacturer, so the lateral epicondyle was aligned with the dynamometer's central axis of rotation (Figure 5.1). Particularly in the group with Achondroplasia, the chair and dynamometer were adjusted to align the lateral epicondyle if needed; additional padding was placed behind the spine to help maintain a static knee angle throughout contractions. The participants' dominant leg was secured with Velcro straps to the chair on the distal portion of the thigh and to the dynamometer around the lower portion of the tibia (~80% tibia length), according to participant comfort. All participants warmed up by performing six continuous submaximal concentric contractions ($60^{\circ}\cdot s^{-1}$) of the KE and knee flexors (KF). Participants then completed a randomised trial of KE iMVCs at 10° intervals, between 60° and 100°, to anatomical zero (where 180° was anatomical zero). Due to the chair being repositioned in the group with Achondroplasia, joint angles were confirmed and recorded using a manual goniometer. Each participant received ~120 seconds rest between each trial. Throughout iMVC trials, participants were verbally encouraged to exert as much force as possible. Visual feedback was also provided to all participants on a monitor. KE and KF iMVCτ values were recorded (2000 Hz) on a computer (Macintosh, iMac,

California) via an A/D converter using an acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, California). The angle that elicited peak KE iMVCτ was used for subsequent analysis.

5.3.2.2 Agonist activation

Agonist activation of during KE iMVCτ production is assessed to observed maximal activation of the muscle and is done so while participants are positioned in the isokinetic dynamometer. Firstly, a counter weight was fixed to the dynamometer to minimise the compliance of the device. To measure agonist activation, two rubber stimulation pads (size ranging from 70 x 90 to 180 x 100 mm; Uni-Patch, MN, USA) were placed proximally and distally along the transverse plane of the dominant femur (Figure 5.1). While in a relaxed state, a percutaneous electrical doublet stimulus (DS7, Digitimer stimulator, Welwyn, Garden City, UK) was passed through the KE at increased increments (~50 mV) and regular intervals (~20 seconds) until a plateau of twitch torque was measured. This supramaximal doublet stimulus was applied to the participants KE (inter-stimulus gap 10 μs and pulse width 50 μs) during KE iMVC. Doublet stimulus has been shown to improve the signal-noise ratio in the assessment of central activation (Belanger and McComas, 1981; Kent-Braun and Ng, 1999). A second doublet was applied approximately 5 seconds after the first stimulus when the muscles were fully relaxed, termed the potentiated doublet. Agonist activation was calculated using the following equation:

$$Equation\ 5.1: Activation\ (\%)\ =\ 100\ \cdot\left(1-\left(\frac{t\ -\ iMVC\tau}{T}\right)\right)$$

Where; t is the interpolated doublet amplitude of the twitch torque, iMVCτ is the isometric maximal voluntary contraction torque and T is the potentiated doublet amplitude (Behm et al., 2001).

5.3.2.3 Measurement of coactivation

Co-activation of the KF was measured in all participants during a KE iMVC, and subsequent KF iMVCτ produced at the angle at which peak KE iMVCτ was measured. In order to determine coactivation of the KF, surface EMG was recorded over the biceps femoris (BF) as it is the largest of the KF group, and is representative of the KF group as a whole (Kellis and Unnithan, 1999). Furthermore, surface EMG was deemed adequate despite the adiposity levels in Achondroplasia (Owen et al., 1990; Hunter et al., 1996a; Hoover-Fong et al., 2007), as a linear relationship is observed in agonist and antagonist EMG between groups of differing adiposity (De Vito et al., 2003). Boundaries of the BF were determined using ultrasonography (Technos MXP Biosound Esaote) to ensure consistent placement of EMG electrodes over the KF. When established two pre-gelled, unipolar, 10 mm, Ag-AgCl percutaneous electromyography (EMG) electrodes (Ambu Neuroline 720, Baltorpbakken, Denmark) were placed distally at ~1/3 of muscle length, to avoid the motor unit of the BF, and ~2 mm apart along the mid-sagittal plane of the muscle (NORAXON, Arizona, USA). A third electrode was placed on the lateral epicondyle of the same femur as a reference (Figure 5.1). Prior to the placement of the electrodes, areas of

the skin were shaved, then cleaned using an alcohol wipe to minimise skin impedance and hence improve the EMG signal. Raw EMG data were recorded at 2000 Hz, with a high and low band-pass filter set at 10 and 500 Hz respectively, and a notch set at 50 Hz. The integral of the root mean square was recorded 0.5 seconds either side of the KE and KF iMVCτ to quantify the level of KF muscle coactivation. Based on a linear relationship occurring between torque and EMG activity (Maganaris et al., 1998), KF torque during KE iMVC was derived by converting the percentage activation of KF EMG during KE iMVC to KF EMG during KF iMVC.

$$Equation\ 5.2: KF\tau = \left(\frac{\left((KE \div KF) \cdot 100\right)}{100}\right) \cdot KF\ iMVC\tau$$

Where KFτ is the KF torque during KE (N·m), KE is the agonist EMG (mV) recorded of the KE during KE iMVC, KF is the antagonist EMG (mV) recorded of the KE during KE iMVC and KF iMVCτ is the torque (N·m) observed during KF iMVC.

The measurement of agonist and antagonist muscle activation are required for the accurate quantification of net KE iMVCτ production, both of which are used in the calculation of specific force (Maganaris et al., 2001; Stebbings et al., 2014). Therefore, net KE iMVCτ was given as the sum of KE iMVCτ and KFτ while a ratio of KF iMVCτ and KE iMVCτ was calculated to describe a balance of quadriceps to hamstring strength.

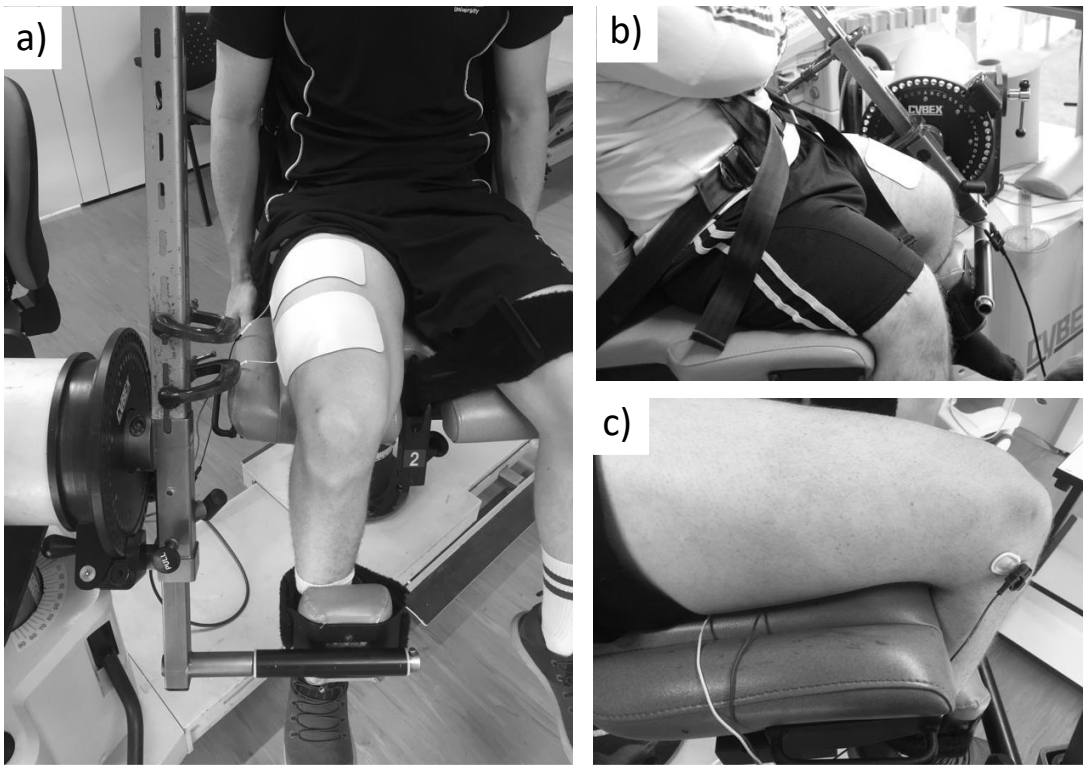

Figure 5.1: Example of the experimental setup of twitch torque activation and counterweight for a) controls and b) an individual with Achondroplasia, and c) the experimental set up for antagonist activation in a control participant.

5.3.2.4 Measurement of muscle volume

To measure VL ACSA, B-mode ultrasonography (Technos MXP Biosound Esaote) was used to obtain a 50 % muscle length transverse plane image of the VL (Reeves et al., 2004c). The origin and insertion of the dominant VL were marked, along with regular intervals of the medial and lateral edges. Muscle length (cm) was determined by the distance between the origin and insertion points with the 50 % percentile marked on the skin. A wire mesh was secured to the skin using non-allergic tape along the transverse plane. The wires were separated ~3 cm apart and ran sagittal to the muscle to act as echo absorbing markers that projected a shadow on the ultrasound

image to act as reference points for analysis (Reeves et al., 2004c), see Figure 5.2. The 5 cm 7.5 MHz linear array probe was placed transversely to the VL with ultrasound transmission gel across the skin. While the probe moved from the medial to the lateral border of the VL, an audio video interleave (AVI) recording with a sampling frequency of 25 Hz (Adobe Premiere Elements version 10, Adobe Systems) was taken. The field of view was set so that anatomical references (femur and aponeurosis between VL and vastus intermedius) were visible at all times. Measurements were taken while the participant was supine and at rest. Individual images (between 5-9), with at least two wire references, were extracted from the recording and used to re-construct the muscle by overlapping the wire and aforementioned anatomical references, on photo editing software (Gimp, Version 2.8.8, GNU Image Manipulation Program). Digitising software (NIH Image J, Version 1.44o, National Institutes of Health, Bethesda, Maryland) was used to measure the ACSA of the VL (Figure 5.3). The volume of the VL was calculated using previously reported constants of MRI regression (Morse et al., 2007a), where:

$$Equation\ 5.3:$$

$$VL\ Volume\ = \left(\frac{-2.9244}{4} + \frac{0.74}{3} + \frac{2.2178}{2} + 0.0244\right) \cdot VL\ length \cdot 50\%\ ACSA$$

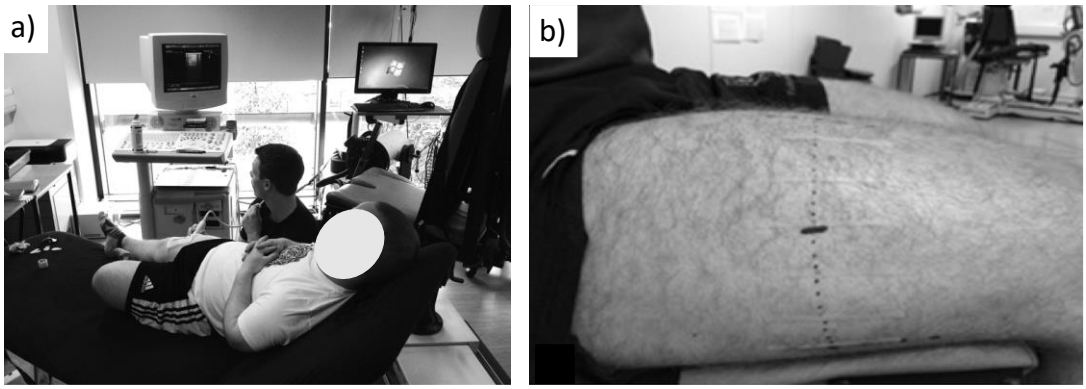

Figure 5.2: Experiment set up of muscle volume estimation for a) an individual with Achondroplasia and b) the identification of the mid-point of the vastus lateralis and the echo absorbing markers along the thigh's transverse plane of a control's leg.

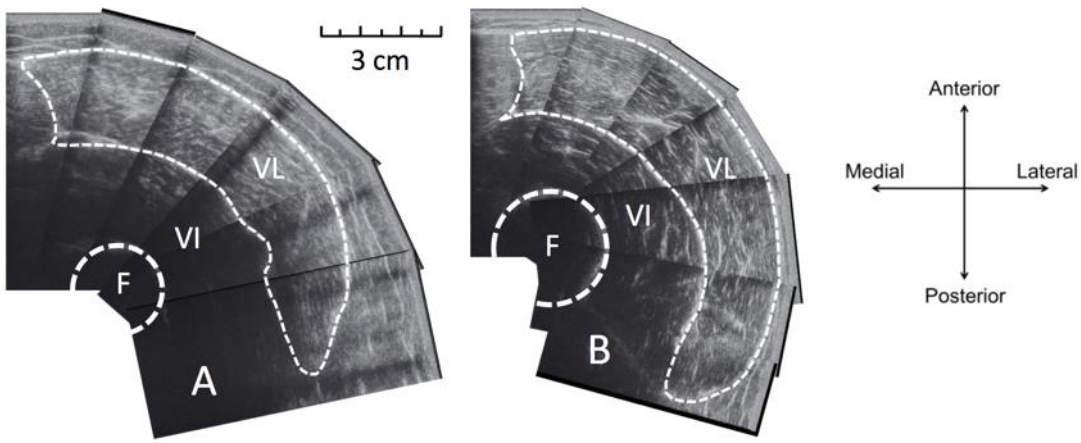

Figure 5.3: An example of a 50% ACSA of scan for a) an individual with Achondroplasia and b) control. VL: Vastus Lateralis; VI: Vastus Intermedius; F: Femur.

5.3.2.5 Muscle architecture

In vivo muscle architecture of the VL was conducted using B-mode ultrasonography (Technos MXP Biosound Esaote) during KE iMVC to observe fascicle length (cm) and pennation angle (θ). The 5 cm, 7.5 MHz linear array probe was held on the mid-sagittal plane on a previously established mid-point of the VL; measured equidistant from the origin-insertion and medial-lateral muscular borders. With water-soluble transmission gel the probe was held against, and at a perpendicular angle to, the skin with minimal pressure. The depth of view was set to ensure a number of fasciculi insertion points and deep aponeurosis were in view (Maganaris et al., 2001). Ultrasound imaging and torque production were synchronised using an external square wave voltage trigger enabling the accurate attainment of iMVC-to-ultrasound. Image recordings were AVI format at a sample frequency of 25 Hz; single images were selected using capture software (Adobe Premiere Elements version 10, Adobe Systems). Images of the VL at rest and iMVC were analysed using digitising software (NIH ImageJ, Version 1.44o, National Institutes of Health, Bethesda, Maryland) whereby fascicle length was determined as the length between the superficial and deep aponeuroses (Narici et al., 1992). Pennation angle was defined as the insertion angle of the fascicle into the deep aponeurosis (Maganaris et al., 2001). With the VL being one of the larger muscles in the body, invariably the dimensions of the probe were not large enough to capture a full fascicle. For these cases, linear extrapolation was used to determine fascicle length as little error (2-7%) is observed at the midpoint of the muscle (Fukunaga et al., 1996; Finni et al., 2003), again using digitising software described above.

5.3.2.6 Physiological cross-sectional area

The PCSA (cm^2) was estimated as the ratio of VL muscle volume to fascicle length (Maganaris et al., 2001), assuming the model used to calculate muscle volume is cylindrical and that the muscle fibres are constant length (Reeves et al., 2004c).

5.3.2.7 Moment arm length

A dual-energy X-ray absorptiometry (DEXA) scanner (Hologic Discovery, Vertec Scientific Ltd, UK), in single energy mode (100 kVp), was used to obtain moment arm length of the patella tendon (PT_{MA}) (Erskine et al., 2014). Participants were asked to lie on their side in a relaxed state. The dominant knee was positioned at the angle acquired from optimal peak force production using a manual goniometer. A single array sagittal plane scan was taken of the knee using a 22.3 x 13.7 cm field of view (Figure 5.4). Obtained scans were exported to and analysed on a Dicom viewer (OsiriZ 5.0.2, Pixmeo Sarl, Geneva, Switzerland). Moment arm length (m) was determined as the perpendicular distance between the estimated tibiofemoral contact point and the posterior aspect of the patella tendon (Tsaopoulos et al., 2006).

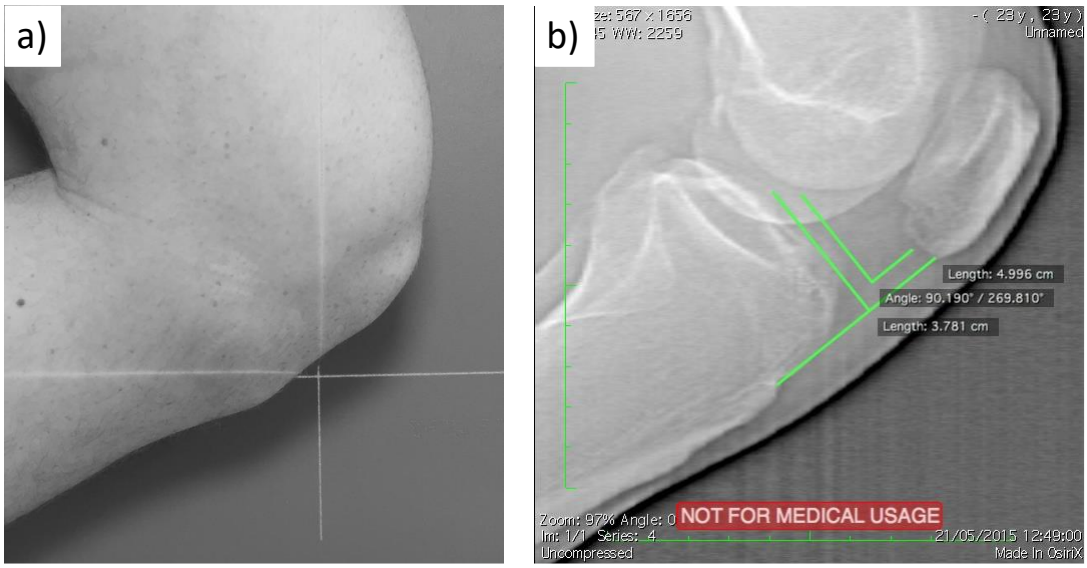

Figure 5.4: a) experimental setup of the sagittal plane single array scan and b) an example of the result and its analysis. Both images are from a control participant.

5.3.2.8 Fascicle force and specific force

To estimate VL fascicle force and in turn specific force the following steps were used:

Patella tendon force (N) was calculated using equation 5.4 (Onambélé et al., 2007):

$$Equation\ 5.4: FPT = \frac{Net\ KE\ iMVC\tau}{MA}$$

Where F_{PT} is the force at the patella tendon (N) during KE iMVC, net KE iMVCτ is calculated above, and MA is the length of the moment arm (m).

Previously reported data show the relative contribution of the VL to the patella tendon to be around 22% (Narici et al., 1992). Equation 5.5 was then used to calculate

VL fascicle force by expressing the VL fascicle force as a ratio of the VL contribution to the cosine of the pennation angle (radians) at KE iMVC.

$$Equation\ 5.5: Fascicle\ Force\ = \frac{VLcon}{cos\theta}$$

Where VL_{Con} is the VL contribution (N) and cos θ is the cosine of pennation at iMVC (radians).

Specific force was represented as the ratio between VL fascicle force and VL PCSA.

5.3.3 Statistical analysis

All data were collated onto a personal computer (Macintosh, MacBook Pro, Apple Computer, Cupertino, California) and analysed using SPSS (v22.0, IBM). Data were confirmed parametric following Shapiro-Wilk and Levene's tests. Independent t-tests were carried out on most measured variables. In addition, Pearson's correlations were performed between related dependent variables. For variables that violated parametric assumptions, a Levene's adjusted P value or a Mann-Whitney U (denoted by * and †, respectively, in Tables 5.1 and 5.2) was performed. Alpha was set at ≤ 0.05 and all results are reported as means (SD).

5.4 Results

The description of participants' anthropometrics are given in Table 2.1 of Chapter 2.

5.4.1 KE and KF iMVCτ

Adult males with Achondroplasia produced 63% less KE iMVCτ than controls (Table 5.1). KF iMVCτ was 82% lower in the group with Achondroplasia compared to controls (Table 5.1). When expressed as a ratio between absolute KE iMVCτ and KF iMVCτ, the group with Achondroplasia produced 49% more iMVCτ from the KE compared to KF than controls (Table 5.2).

5.4.2 Activation and coactivation

There was no difference in maximal activation between groups, but the group with Achondroplasia had a 70% greater coactivation of the BF during KE iMVC compared to controls (Table 5.1).

5.4.3 Net KE iMVCτ

Both groups increased KE iMVC by ~6% when corrected for BF coactivation (Table 5.1). The net KE iMVCτ produced by the VL was 63% less in the group with Achondroplasia compared to controls (Table 5.1). There was no significant correlation between body fat percentage and net KE iMVCτ in the group with Achondroplasia ($r = 0.110$, $P = 0.763$) or controls ($r = 0.411$, $P = 0.090$).

5.4.4 Morphology and architecture

The group with Achondroplasia had 41% smaller VL length than controls (Table 5.1). VL morphology differed between groups as the group with Achondroplasia had a 20% smaller ACSA (Table 5.1, Figure 5.5) and a 53% smaller VL muscle volume than controls (Table 5.1). The group with Achondroplasia exhibited a 17% greater pennation angle but 17% smaller fascicle length during KE iMVC than controls (Table 5.1). PCSA was 42% smaller in the group with Achondroplasia compared to controls (Table 5.1). Correlations revealed no significant relationship between VL muscle volume and net KE iMVCτ production in the group with Achondroplasia (R^2 = 0.056, P = 0.508, Figure 5.5), whereas for the same variables in controls, a significant relationship did exist (R^2 = 0.286, P = 0.022, Figure 5.5). Despite the diverging regression lines, a Z-transformation showed the slopes between groups were similar (P = 0.442).

The group with Achondroplasia produced 53% less force per unit area compared to controls when presenting KE iMVCτ as a ratio to ACSA (Table 5.2). When net KE iMVCτ is expressed as a ratio to total-body mass, the group with Achondroplasia again display a 43% reduction to controls (Table 5.2). The group with Achondroplasia displayed a 67%, reduction in net KE iMVCτ when presented as a ratio to fat free mass (Table 5.2). There was no relationship between ACSA and PCSA (R^2 = 0.016, P > 0.05) for the group with Achondroplasia, whereas a significant relationship for the same variables was observed for controls (R^2 = 0.254, P = 0.032).

5.4.5 Force measurements

The length of the PT_{MA} were similar between groups (Table 5.1). Patella tendon force (60%), fascicle force (59%) and specific force (29%) were all lower in group with Achondroplasia compared to controls (Table 5.1).

Table 5.1: Morphological and functional characteristics of the vastus lateralis in controls and adults with Achondroplasia adults. Values presented as mean (SD).

	Control	Achondroplasia	P value
iMVCτ KE (N·m)	256 (47)	95 (24)	< 0.001
iMVCτ KF (N·m) *	105 (19)	19 (7)	< 0.001
Activation (%) *	92.0 (5.9)	83.9 (13.9)	0.105
Coactivation (%) *	12.6 (5.3)	42.6 (20.0)	0.001
Net iMVCτ (N·m) †	287 (49)	106 (26)	< 0.001
Volume (cm^3) *	604 (102)	284 (36)	< 0.001
Fascicle Length (cm) *	8.2 (1.5)	6.8 (1.5)	0.027
ACSA (cm^2) *	27.7 (4.4)	22.2 (2.6)	< 0.001
Pennation Angle (°) †	17.4 (2.4)	20.9 (4.6)	0.027
Muscle Thickness (cm)	28.4 (7.6)	20.6 (8.3)	0.550
PCSA (cm^2)	74.7 (13.7)	43.2 (9.9)	< 0.001
Moment Arm (m) †	0.040 (0.002)	0.037 (0.005)	0.309
Patella Tendon Force (N)	7296 (1319)	2930 (974)	< 0.001
VL Fascicle Force (N)	1704 (303)	702 (235)	< 0.001
Specific Force ($N \cdot cm^{-2}$) †	23.6 (6.4)	16.7 (6.0)	0.014

iMVCτ, isometric maximal voluntary contraction torque; ACSA, anatomical cross-sectional area; PCSA, physiological cross-sectional area. * adjusted P value following Levene's; † Mann Whitney-U.

Table 5.2: Morphological and functional characteristics of the vastus lateralis normalised anatomical structures in controls and adults with Achondroplasia. Values presented as mean (SD).

	Control	Achondroplasia	P Value
iMVCτ KE:KF (%)	41.1 (9.2)	20.2 (6.7)	< 0.001
VL Length:Stature (%) †	18.8 (0.8)	14.3 (0.7)	< 0.001
TBM:Volume ($kg \cdot cm^{-3}$)	7.76 (1.17)	4.65 (0.69)	< 0.001
Net iMVCτ:ASCA ($N \cdot m \cdot cm^{-2}$)	2.14 (0.37)	2.81 (0.73)	0.003
Net iMVCτ:TBM ($N \cdot m \cdot kg^{-1}$)	3.72 (0.71)	1.71 (0.28)	< 0.001
Net iMVCτ:FFM ($N \cdot m \cdot kg^{-1}$) †	4.99 (0.78)	2.54 (0.43)	< 0.001
Net iMVCτ:Volume ($N \cdot m \cdot cm^{-3}$)	0.48 (0.08)	0.38 (0.10)	0.006
PT Moment arm:VL Length (cm) †	11.78 (0.96)	19.07 (3.25)	< 0.001
Net iMVCτ:PSCA ($N \cdot m \cdot cm^{-2}$)	3.96 (0.99)	2.55 (0.80)	0.001

VL, vastus lateralis; TBM, total-body mass, iMVCτ, isometric maximal voluntary contraction torque; ACSA, anatomical cross-sectional area; PCSA, physiological cross-sectional area; PT, patella tendon. † Mann Whitney-U.

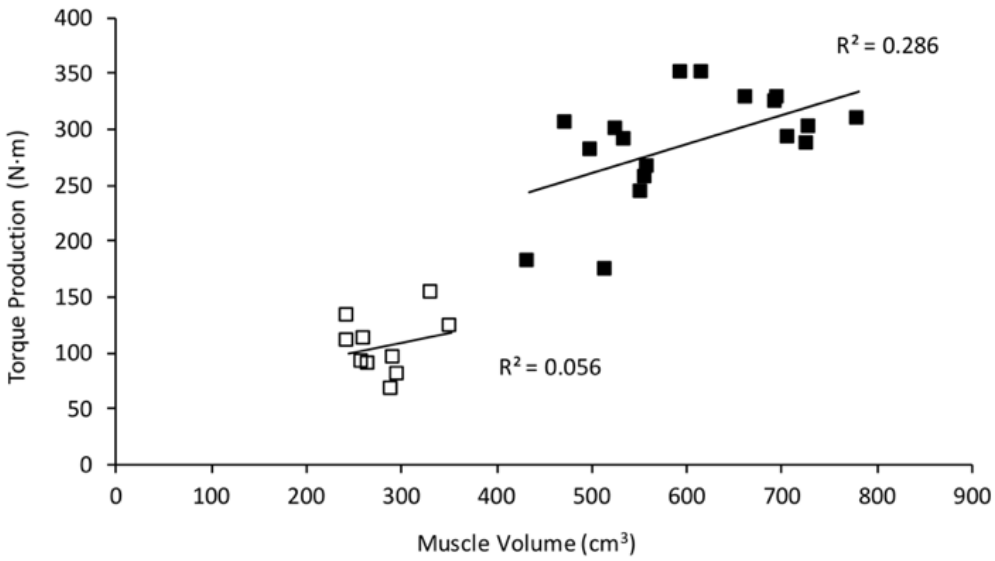

Figure 5.5: Scatter plot showing the relationship between VL muscle volume (cm^3) and torque production (N·m) for the group with Achondroplasia (open) and controls (closed). Trend lines including R^2 are also given for each group respectively.

5.5 Discussion

This Chapter aimed to assess the *in vivo* muscle morphology, KE iMVCτ production and specific force of the VL in adults with Achondroplasia and age-matched healthy adults. The main findings were 1) net KE iMVCτ, VL ACSA, volume and PCSA were lower in adults with Achondroplasia compared to controls; 2) differences in net KE iMVCτ were not accounted for by the differences in muscle size; 3) KF coactivation was higher in the group with Achondroplasia than controls, and; 4) when morphological, architectural, neurological and biomechanical differences were accounted for, a 29% smaller specific force was observed in the group with Achondroplasia.

A large portion of neuromuscular function research describes the relationship between muscle size and force production, suggesting that muscle size is the predetermining factor for muscle strength (Maughan et al., 1983; Bruce et al., 1997; Tonson et al., 2008; Stebbings et al., 2014). Groups of shorter statures consistently present with smaller muscle size and lower MVC strength than their taller counterparts (Sartorio and Narici, 1994; Bottinelli et al., 1997; Y. J. H. Janssen et al., 1999; Morse et al., 2008; O'Brien et al., 2010a; O'Brien et al., 2010c); when iMVCτ is normalised to muscle size, differences between control and short stature groups are accounted for (Sartorio and Narici, 1994; Bottinelli et al., 1997; Y. J. H. Janssen et al., 1999). The data from the present study is partially consistent with these previous findings. The group with Achondroplasia were 82% weaker than controls in terms of KE iMVCτ, however this was not entirely accounted for by ACSA which was only 20%

smaller. It is likely therefore that architectural and neurological factors contribute to weakness in individuals with Achondroplasia. It should be noted however, that despite accounting for these factors, a deficit in specific force between groups remains. This could be subsequently attributed to physiological and/or biomechanical factors between groups or methodological measures of specific force, as discussed below.

5.5.1 Muscle morphology in individuals with Achondroplasia

The extent of group differences in muscle size between the observed groups was not consistent for each variable. For example, a 20% smaller VL ACSA in the group with Achondroplasia underestimated the difference in PCSA which was 42% smaller than controls. This was due to the smaller muscle length and hence smaller VL volume in the group with Achondroplasia compared to controls. ACSA must therefore be considered an inaccurate method of assessing contractile area between groups of heterogeneous muscle length such as presented here.

Although PCSA is the closest approximation to sarcomeres in parallel and therefore contractile area (Lieber and Friden, 2000), it is possible that PCSA may be overestimated in the group with Achondroplasia. The over estimation of PCSA is likely due to the differences in architectural properties at iMVC between groups. In controls, increased tendon compliance (i.e. more strain when under a relative stress) alters muscle architecture at iMVC, with increased pennation angle, fibre shortening and a leftward shift in the length tension relationship is observed (Maganaris et al.,

2001; Reeves et al., 2003a; Reeves, 2006). Only a larger pennation angle at iMVC was observed between groups in the present study, as resting fibre length not measured. Assuming the patella tendon of individuals with Achondroplasia is more compliant than controls, given the related observations in contractile properties with altered tendon compliance (Reeves, 2006), there is likely to be a greater fibre length of the group with Achondroplasia at iMVC compared to controls. PCSA is therefore overestimated as PCSA = ACSA ÷ fibre length. Given that PCSA is the denominator when calculating specific force, a large PCSA (with the same fascicle force) equates to a lower specific force. Were the fibre lengths of the group with Achondroplasia 17% shorter and 17% more pennate at iMVC than controls, but the fibre angle remained the same between groups at KE iMVC, the fibre length of the group with Achondroplasia would be 9% longer than that presented. This would result in a 47% smaller PCSA compared to controls, 5% more than the measured values. This consequently leads to a 15% lower specific force in the group with Achondroplasia compared to controls.

The difference in muscle architecture at iMVC between groups therefore appears to contribute to the difference in specific force and may be owing to a more compliant patella tendon of the group with Achondroplasia. However, there appears to be no quantitative measure of tendon compliance in any population with Achondroplasia within the literature to confirm this. Furthermore, this theory may only explain some of the 23% difference in specific force between groups.

5.5.2 Specific force

Specific force provides an accurate representation of the *in vivo* contractile properties of the whole muscle and has been used to described the force characteristics of numerous different cohorts and muscle groups (Narici et al., 1992; Degens et al., 1995; Fukunaga et al., 1996; Maganaris et al., 2001; Morse et al., 2007b; Morse et al., 2008; Erskine et al., 2009; O'Brien et al., 2010a; O'Brien et al., 2010c; Stebbings et al., 2014). Recently it has been shown that inter-individual variability in the measurements of specific force alludes to the fact that population variance in specific force may be due to a lower fibre specific force (i.e. myofilament differences), or an overestimation of muscle area through the inclusion of non-contractile material in the measurement of muscle mass (Stebbings et al., 2014). Several research groups have investigated specific force at the myofilament level to identify intramuscular differences (Trappe et al., 2000; Urbanchek et al., 2001; Trappe et al., 2003). With no apparent measure of force production made at the myofilament level in individuals with Achondroplasia, the lower specific force observed in the group could be due to the collagenous defect. That is, as the collagen formation in the endplates of the bone is different between individuals with Achondroplasia and controls, the collagenous connective tissue in muscle may alter the protein structures at the myofilament level. This theory though, is speculative as there appears to be no reports of muscle structure at the myofilament level in individuals with Achondroplasia.

It is possible that the presentation of a lower specific force could be due, in part, to an overestimation of muscle size owing to the use of ultrasound to measure ACSA.

Ultrasound, as with MRI, requires the measurement of the area encapsulated by aponeuroses to determine ACSA. The area within these limits includes muscle, connective tissue and fat infiltration. Previous reports (Hecht et al., 1988; Owen et al., 1990; Hoover-Fong et al., 2007) and in this thesis (Chapter 2, Table 2.1) show that individuals with Achondroplasia have higher body fat percentage than controls. The fibroblast mutation that causes Achondroplasia may also play some part in connective tissue distribution within the muscle, although this is at present unreported. Therefore, the measured ACSA of the VL in individuals with Achondroplasia may reflect a "pseudohypertrophy" due to the probable higher amount of intramuscular fat infiltration, as observed in people with higher body fat (Tomlinson et al., 2014a). This pseudohypertrophy would increase muscle volume and PCSA measurement, with no change in contractile mass and in turn lower the calculation of specific force in individuals with Achondroplasia; it is important to note here that this methodological limitation is present in all populations and conditions. Regardless of these methodological discrepancies, when scaling strength and muscle size, a lower specific force persists in the present group with Achondroplasia, which could be attributed to either an infiltration of non-contractile material (i.e. fat), differences in single fibre properties or differences in tendon properties compared to controls.

5.5.3 Coactivation and moment arm length

In this study, the use of DEXA to measure PT_{MA} led to two important observations of the knee in the group with Achondroplasia. Firstly, there appears to be a lower joint

congruency between femur and tibia in the knees with Achondroplasia (Figure 5.6), agreeing with observations made by Aykol et al. (2015). The apparent reduced tibiofemoral joint congruency in individuals in Achondroplasia would likely reduce tibiofemoral joint stability. In clinical, injured and juvenile populations, where joint congruency is lower, higher coactivation of the BF is observed during KE (Fairbank et al., 1984; Kellis and Unnithan, 1999; Kellis, 2003). In the present study, the group with Achondroplasia had a 70% higher coactivation of the BF during KE iMVC compared to controls. Therefore, the higher coactivation of the BF during KE iMVC in the group with Achondroplasia is likely due to the reduced tibiofemoral joint congruency and may act as an injury prevention mechanism. In this scenario, the hamstrings of individuals with Achondroplasia are activating during KE to reduce the anterior movement of the tibia in relation to the femur. This would protect ligamentous structures in the knee, such as the anterior cruciate ligament. It is possible that this mechanism exists in other muscle groups and joints around the body of an individual with Achondroplasia. The higher coactivation of hamstrings, and possible other muscles in individuals with Achondroplasia may also influence activities of daily living, such as metabolic cost (as observed in Chapter 4). There is however, a lack of comparative data expressing the activation profiles of the muscles in individuals with Achondroplasia during different intensities of contraction to expand on the theories presented. Therefore, the suggestions made from the current findings warrant further work.

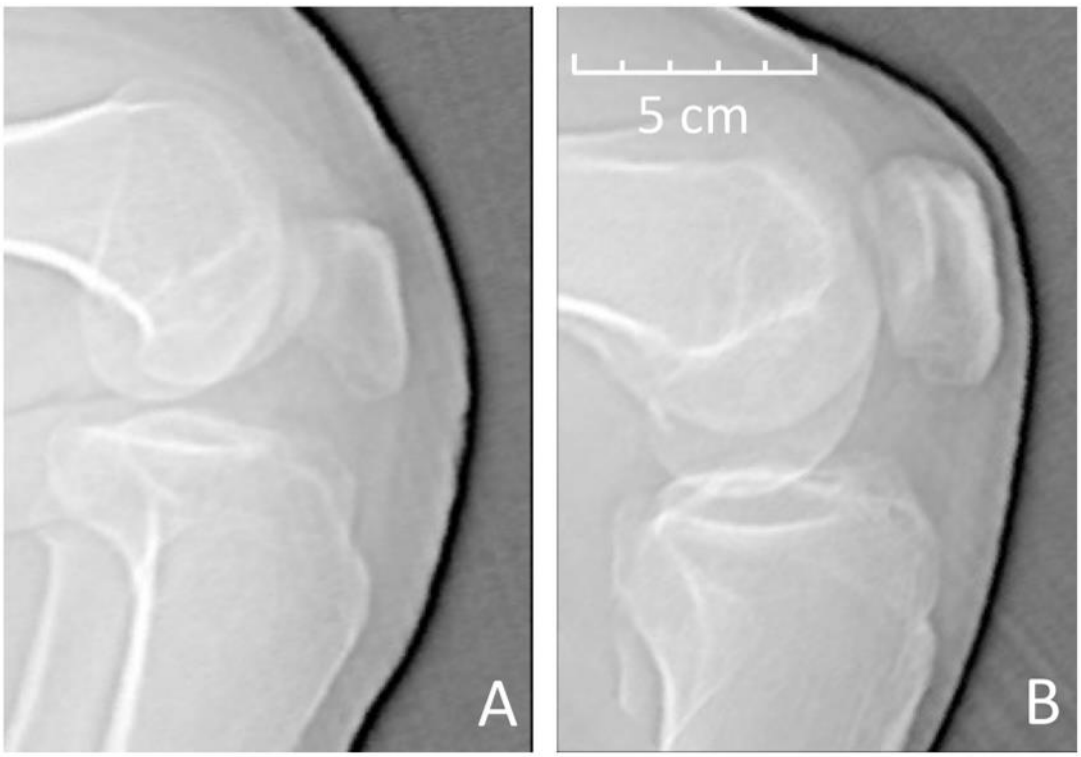

Figure 5.6: Sagittal knee scans of the femoral contact point for an a) an individual with Achondroplasia and b) control participant. Note: the visible lower femoral contact point with the tibia in Figure 5.6a.

The second observation from DEXA scan was that the absolute PT_{MA} between groups was the same, meaning that individuals with Achondroplasia have a longer PT_{MA} relative to the femur (here measured as VL length). This finding is different to other shorter statured groups who show a proportionally smaller moment arms compared to taller statured individuals (Morse et al., 2008). The relatively larger PT_{MA} in the group with Achondroplasia likely aids KE torque production, despite the 63% lower net KE iMVCτ compared to controls. For example, were the PT_{MA} of the current group with Achondroplasia to be proportionally smaller to their femur length (i.e. 37% shorter), they would have produced 76% less net KE iMVCτ than controls. Whilst PT_{MA}

appears to aid torque production in individuals with Achondroplasia, PT_{MA} changes during KE (Baltzopoulos, 1995; Maganaris et al., 1998; Maganaris et al., 1999) which leads to differences in force production (Tsaopoulos et al., 2006). In the present study, PT_{MA} was measured at rest and did not account for changes of PT_{MA} during contraction. It was assumed that the changes in PT_{MA} during KE iMVC would be similar between groups as it is unreported if the same changes in PT_{MA} occur in individuals with Achondroplasia during KE. Any change in the PT_{MA} of individuals with Achondroplasia during contraction may further aid or hinder torque production of the group, but this is yet to be observed. The presented data from this study appears to be the only data that accounts for the moment arm of joints with Achondroplasia during contraction in any joint.

5.6 Conclusion

This is the first study, to the author's knowledge, that has systematically accounted for various physiological and biomechanical modulators of force production in individuals with Achondroplasia. The main finding is that the group with Achondroplasia produced 29% less specific force than controls. These results may only explain the variance in muscle morphology as further work into methodological validity of measuring specific force in, and myofilament descriptions of individuals with Achondroplasia specific force is needed to further these data.

Chapter 6: Morphological and mechanical properties of the human patella tendon in adults with Achondroplasia

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6.1 Abstract

Achondroplasia is a genetic mutation of fibroblast growth factor receptor resulting in impaired growth plate development in long bones due to lower collagen turnover. Despite the characteristic shorter stature and lower strength in groups with Achondroplasia, little is known of the tendon's mechanical properties under contraction. The aim of this study was to therefore measure the mechanical properties (stress, strain, stiffness and Young's Modulus) of the patella tendon (PT) in 10 adults with Achondroplasia (22 $\pm$3 yrs) and 17 male controls (22 $\pm$2 yrs) at isometric maximal voluntary contraction (iMVC) using ultrasonography. The group with Achondroplasia produced 54% less stress at iMVC than controls ($P < 0.001$). Maximal excursion of the PT was 22% less at iMVC in the group with Achondroplasia compared to controls ($P < 0.001$), but there was no difference in strain between groups ($P > 0.05$). The PT were 47% less stiff ($P < 0.001$) and had a 51% lower Young's modulus at iMVC ($P < 0.001$) in the group with Achondroplasia compared to controls. The PT of individuals with Achondroplasia are indeed more compliant than controls which may contribute to lower relative force production. The causes of this higher compliance are unclear but are likely due to the collagen related genetic mutation which causes Achondroplasia.

6.2 Introduction

The previous Chapter observed a lower specific force of the vastus lateralis (VL) in adults with Achondroplasia compared to age matched adults of average stature (controls). The lower specific force may be accounted for by the methods used to measure specific force, myofilament differences between groups, or the mechanical properties of the patella tendon between groups. Given the stature and leg length differences between groups (Chapter 2) there is likely to be a difference in patella tendon length. While fibroblast growth factor receptor (FGFR3) is linked unequivocally with bone development, due to the collagenous association, it is possible that the mutation is likely to alter other collagenous structures, such as the tendon, but the mechanical properties of any tendon are yet to be reported empirically for individuals with Achondroplasia.

For groups of shorter statures, such as individuals with Achondroplasia, it would be appropriate to acknowledge the probable down-scaling of tendon morphology as well as the properties of the tendon during muscular contraction. For example, the shorter patella tendon length in children compared to adults can be normalised entirely by the proportionally smaller morphological differences of the tendon (O'Brien et al., 2010b). During loading though, both the force production passing through the tendon and the amount of lengthening the tendon goes through contribute to the tendon's ability to transfer force from muscle to bone (Maganaris and Paul, 2000a; Hewett et al., 2005; Arampatzis et al., 2006; Onambélé et al., 2006; Reeves, 2006; Fletcher et al., 2010). A tendon that lengthens more for a given force production is more compliant and less effective at transferring force to the bone, due

in part to the characteristics of fascicle shortening (Reeves, 2006). Whilst tendon loading and excursion during contraction have been described for differing human populations, both *in vivo* and *in vitro*, there remain no data for populations with Achondroplasia.

As observed in the previous Chapter, isometric maximal voluntary contraction torque (iMVCτ) of the knee extensors (KE) was lower in adults with Achondroplasia compared to controls. Furthermore, a lower force was recorded at the patella tendon (F_{PT}) in the group with Achondroplasia compared to the control group. Force measured at a tendon can be normalised to its cross-sectional area (CSA) and presented as stress (tendon force/tendon CSA). In children, CSA of the patella tendon (CSA_{PT}) helps scale absolute measures of F_{PT} when compared to adults, suggesting that tendon volume is proportional to stature (O'Brien et al., 2010b). In disproportionately sized groups, such as those with Achondroplasia, there may well be a scaling factor of F_{PT} to CSA_{PT}, but the magnitude of scaling is currently unreported. While stress is the normalised tendon force, strain is normalised tendon excursion. Defined as the ratio of length change to resting length, strain is a dimensionless number that provides a relative difference in tendon extensibility. For groups such as children, this is useful given the proportionally shorter tendons and shows children's tendons deform more under the same loading as adults (O'Brien et al., 2010b); as with stress though, no strain measures have been reported in the tendons of individuals with Achondroplasia.

The ratio of stress and strain gives Young's Modulus which provides the material properties of the tendon (Maganaris, 2002; Onambélé et al., 2007; Onambélé et al., 2006; Reeves, 2006; Seynnes et al., 2009). The observations of Young's Modulus made in child tendons suggest that that there are intrinsic differences between child and adult tendons. Given the likely smaller, but unknown scaling differences, of tendon morphology between individuals with Achondroplasia and controls the calculation of Young's Modulus would help to firstly normalise mechanical properties of the patella tendon between individuals with Achondroplasia and controls and thereafter infer the intrinsic properties of the patella tendon.

The aim of this study was therefore to measure the *in vivo* material properties of patella tendon in adults with Achondroplasia and compare them to controls.

The objectives of this chapter were to:

1) measure the *in vivo* excursion of the patella tendon in both groups during maximal voluntary contraction using ultrasonography;
2) measure the *in vivo* contractile properties of the vastus lateralis during maximal voluntary contraction in both groups using ultrasonography;
3) Measure the *in vivo* mechanical properties of both groups' patella tendon through the measurement of Young's Modulus.

6.3 Method

6.3.1 Participants

Ten adults with Achondroplasia and 17 age matched controls that were free from lower limb injury volunteered to participate in the study and are described in Table 2.1 in Chapter 2.

6.3.2 Patella tendon cross sectional area

While at rest participants sat in an isokinetic dynamometer with their dominant leg strapped into the lever arm so that 90° of knee flexion was attained (180° = full extension). Patella tendon origin and insertion were identified using ultrasonography (Technos MXP Biosound Esaote, UK) by holding a 5 cm 7.5 MHz linear array probe in the sagittal plane to the patella tendon. Positions were marked on the skin and measured using a tape measure. Intervals (25, 50 and 75%) of tendon length were measured and marked transversely. CSA_{PT} was taken by applying water-soluble transmission gel to the probe and placing along the sagittal plane of the patella tendon at the marked percentages of patella length with minimal pressure. Depth of view was such that medial and lateral borders of the patella tendon were viewable. Image recordings were AVI format at a sample frequency of 25 Hz; single images were selected using capture software (Adobe Premiere Elements version 10, Adobe Systems) and analysed using digitising software (NIH ImageJ, Version 1.44o, National Institutes of Health, Bethesda, Maryland). High reliability has been shown measuring patella tendon length (Skou and Aalkjaer, 2013) and CSA_{PT} with ultrasound (Gellhorn

and Carlson, 2013). Patella tendon volume was calculated using the truncated cone method (Figure 6.1):

$$Equation\ 6.1.1: R = \sqrt{\frac{A}{\pi}}$$

$$Equation\ 6.1.2: TCV = \frac{\pi}{3} \cdot \left(L_{PT} \cdot \left(R12 + R22 + (R1 \cdot R2)\right)\right)$$

$$Equation\ 6.1.3: CV = \frac{(A \times L_{PT})}{3}$$

$$Equation\ 6.1.4: Tendon\ Volume = CVprox + TCV1 + TCV2 + CVdist$$

Where R is the radius (cm) of a given scan, A is the measured CSA_{PT} (cm^2), TCV is the mid-proximal (TCV_1) and mid-distal (TCV_2) truncated cone volumes (cm^3) representing 25-50% and 50-75% of the tendon respectively, L_{PT} is patella tendon length (cm) between two measured points, R_1 and R_2 are the radii (cm) of two scans respectively, while CV is cone volume (cm^3) of the proximal (CV_{prox}) and distal cone (CV_{dist}) representing 0-25% and 75-100% of the tendon respectively. Tendon volume (cm^3) is the sum of all inter-scan volumes.

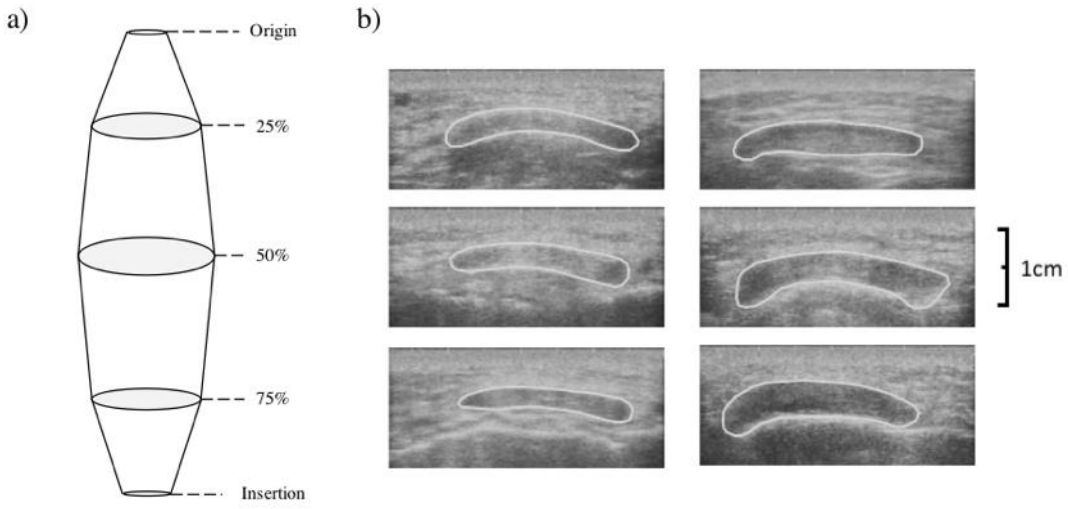

Figure 6.1: a) Schematic depicting the calculation of tendon volume using the truncated cone method and b) transverse ultrasound scans of the patella tendon CSA at 25% (top), 50% (middle) and 75% (bottom) of patella tendon length for an individual with Achondroplasia (Left) and control (Right) participant.

6.3.3 Knee extensor torque measurements

iMVCτ of the dominant KE (Achondroplasia N = 9/10 right leg, control N = 16/17 right leg) were recorded using an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA). Participants were seated upright with the dynamometer and chair positioned in accordance with the calibration guidelines, so the lateral epicondyle was aligned with the dynamometer's central axis of rotation (see Figure 5.1 in Chapter 5). Particularly in the group with Achondroplasia, the chair and dynamometer were adjusted to align the medial malleolus if needed. Additional padding was placed behind the spine to stabilise the individual to ensure the knee maintained the set position throughout contractions. Velcro straps were used to

secure the dominant leg to the chair via the distal portion of the thigh, while the lever arm was attached to the tibia at ~80% of its length. Participants warmed up by performing six continuous submaximal concentric contractions of the KE and knee flexors (KF). To reduce the effect of creep in the patella tendon, participants completed four KE iMVCs at 90° flexion (180° = full extension) with ~120 seconds rest between trials (Pearson et al., 2007). Following warm up, two iMVC trials were recorded with participants verbally encouraged to exert as much force as possible. A ramped MVC, lasting ~5 s, was instructed with visual feedback provided to all participants, iMVC was assumed based upon a visible plateau of KE torque on a monitor. KE torque values were recorded (2000 Hz) on a computer (Macintosh, iMac, Apple Computer, Cupertino, California) using an acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, California).

6.3.4 Vastus lateralis architecture and fascicle displacement

The origin and insertion of the VL were identified using B-mode ultrasonography (Technos MXP Biosound Esaote) by holding the probe along the transverse plane of the muscle. Upon identification of landmarks, a tape measure was used to determine VL length. *In vivo* muscle architecture of VL was measured using B-mode ultrasonography during the last warm up KE iMVC to observe fascicle length (cm) and pennation angle (θ). Both fascicle length and pennation were measured during rest and iMVC to observe the change in each respective variable (Figure 6.2). The same linear array probe described above was held on the mid-sagittal plane on a previously established mid-point of the VL; measured equidistant from the origin-insertion and

medial-lateral muscular borders. With water-soluble transmission gel, the probe was held normal to the skin with minimal pressure. View depth was set to ensure a number of fasciculi insertion points and deep aponeurosis were in view (Maganaris, 2001). Imaging and torque production were synchronised by an external voltage trigger enabling the accurate attainment of iMVC-to-ultrasound. Image recordings were AVI format at a sample frequency of 25 Hz; single images were selected using capture software (Adobe Premiere Elements version 10, Adobe Systems). Images of the VL at rest and iMVC were analysed using digitising software (NIH ImageJ, Version 1.44o, National Institutes of Health, Bethesda, Maryland). Fascicle length was determined as the distance between the superficial and deep aponeuroses along a visible fascicle (Maganaris, 2001). Pennation angle was defined as the insertion angle of the fascicle into the deep aponeurosis (Maganaris, 2001). With the VL being one of the larger muscles in the body, invariably the dimensions of the probe were not large enough to capture a full fascicle, for these cases linear extrapolation was used to determine fascicle length as little error (2-7%) is observed at the midpoint of the muscle (Fukunaga et al., 1996; Finni et al., 2003), again using digitising software described above.

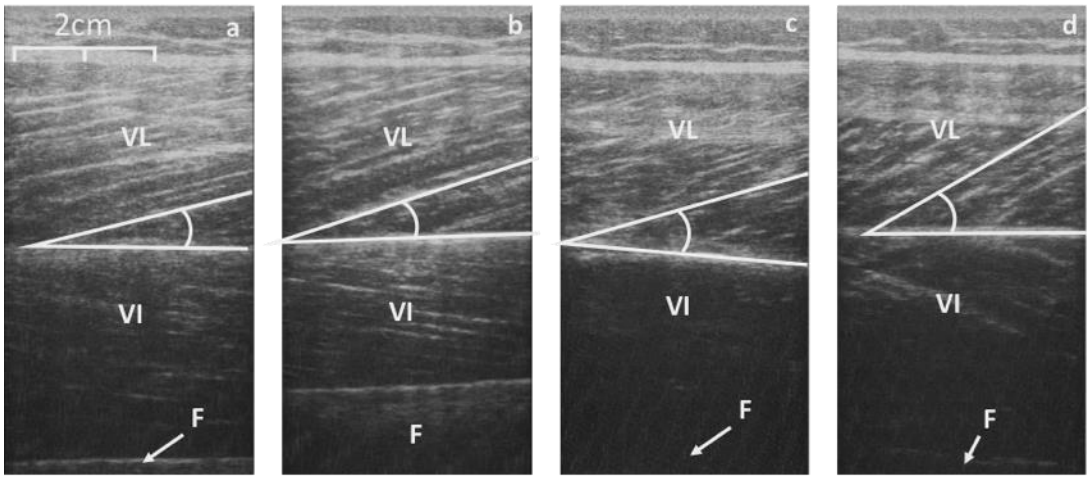

Figure 6.2: Sagittal scans of the VL at rest (a = control, c = Achondroplasia) and at iMVC (b = control, d = Achondroplasia). Deep aponeurosis is highlighted along with a fascicular insertion. VL, Vastus Lateralis; VI, Vastus Intermedius; F, Femur.

6.3.5 Tendon elongation measurements

Tendon elongation was observed from rest to iMVC. 50% of the measured resting tendon length described above was used to place a thin (~10 mm) echo absorbing marker (Micropore tape) on the skin, across the tendon, to act as a reference marker (Figure 6.3). The ultrasound probe was held in the sagittal plane over the patella tendon so that the reference marker was identifiable with both the patella tendon origin (proximal, trial 1) and the patella tendon insertion (distal, trial 2) in the same image. Ultrasound images were then stitched together using digitising software (GIMP) and analysed using Image J. High reliability (r = 0.910) of tendon excursion using this method is reported elsewhere (Onambélé et al., 2007). Participants were instructed to perform a ramped iMVC where the test was terminated once a plateau of torque trace was observed on a monitor. Ultrasound imaging and torque production were synchronised using an external voltage trigger enabling the

accurate attainment of iMVC-to-ultrasound. As described by Onambélé et al. (2007), analysis of the images were completed after the calculation of torque at 10% intervals of iMVC, where excursion was determined from the respective origin and insertion of the tendon to the respective superior and inferior edge of the reference marker (Figure 6.4). At each 10% interval of iMVC, the displacement of the patella tendon from the respective trial 1 and trial 2 measurements were summed with the individual measurement of echo absorbing tape to calculate tendon length, as described elsewhere (Onambélé et al., 2007). Elongation of the patella tendon was recorded once, but to ensure intrascan reliability, images were digitised twice on separate occasions. These values were then used to determine total strain described below.

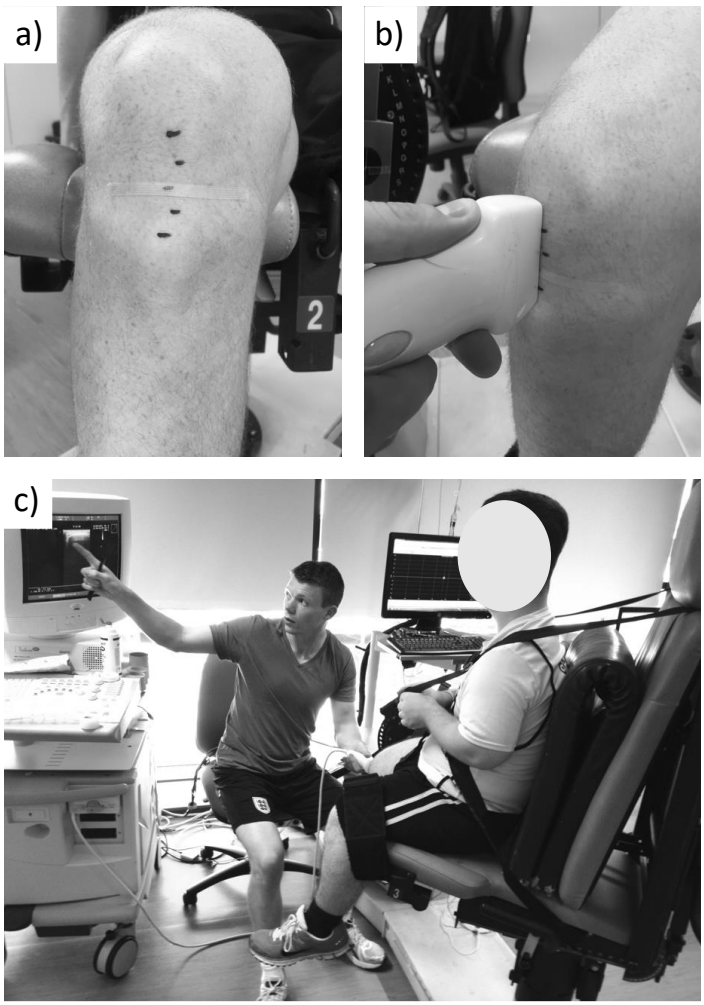

Figure 6.3: a) proximal (top) and distal (bottom) markers that identify absolute and proportionally patella tendon lengths for a control participant, b) ultrasound probe placement that observes the proximal and reference marker (echo absorbing tape) for a control participant, and c) a participant with Achondroplasia in the experimental set up with a feedback monitor.

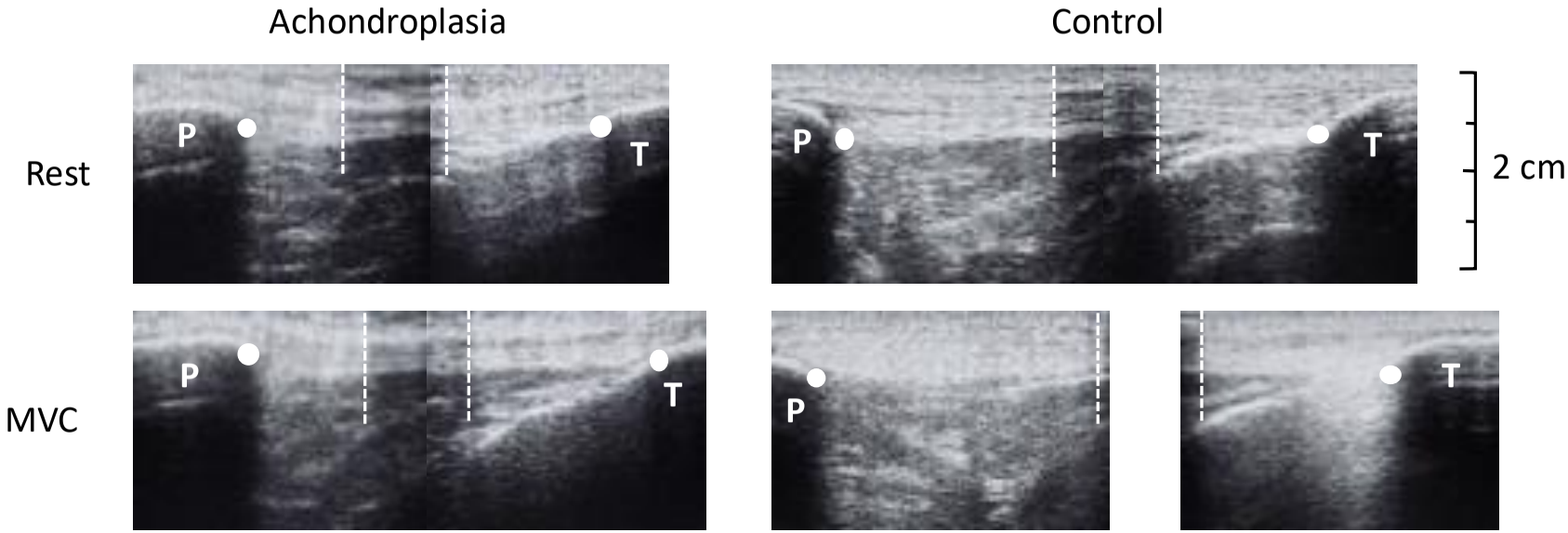

Figure 6.4: Ultrasound images during patella tendon elongation measures from rest (top) to maximal voluntary contraction (bottom) in an individual with Achondroplasia (left images) and control (right images). White dots represent the measurement of tendon length from patella tendon origin (patella, P) and insertion (tibia, T). For the longer tendons that exceed the ultrasound probe's field of view, an echo-absorptive marker was used as a reference point to help stitch images together (Onambélé et al., 2007). The boundaries of the marker are identified by the white dotted lines.

6.3.6 Agonist activation

To accurately measure KE iMVCτ, agonist activation was assessed to estimate the degree of total activation of the KE. To do so, two rubber stimulation pads (size ranging from 70 x 90 to 180 x 100 mm; Uni-Patch, MN, USA) were placed proximally and distally along the transverse plane of the dominant femur. A counter weight was fixed to the dynamometer to minimise the compliance of the device. While in a relaxed state, a percutaneous singlet electrical stimulus (DS7, Digitimer stimulator, Welwyn, Garden City, UK) was passed through the KE at increasing increments (~50 mV) and regular intervals (~20 seconds) until a plateau of twitch torque was measured. A supramaximal doublet stimulus was subsequently applied to the participant's KE (interstimulus gap 10 μs and pulse width 50 μs) during KE iMVC. Doublet stimulus has been shown to improve the signal-noise ratio in the assessment of central activation (Belanger and McComas, 1981; Kent-Braun and Ng, 1999). A second doublet was applied approximately 5 seconds after the first stimulus when the muscles were fully relaxed, termed the potentiated doublet (Behm et al., 2001). Agonist activation was calculated using the following equation:

$$Equation\ 6.2: Activation\ (\%) = 100 \cdot \left(1 - \left(\frac{t - iMVC\tau}{T}\right)\right)$$

Where; t is the interpolated doublet amplitude of the twitch torque, iMVCτ is the isometric maximal voluntary contraction torque and T is the potentiated doublet amplitude (Behm et al., 2001).

6.3.7 Measurement of coactivation

Co-activation of the KF was measured in all participants during a KE iMVC, and subsequent KF iMVCτ produced at the angle at which peak KE iMVCτ was measured. In order to determine coactivation of the KF, surface EMG was recorded over the biceps femoris (BF) as it is the largest of the KF group, and is representative of the KF group as a whole (Kellis and Unnithan, 1999). Furthermore, surface EMG was deemed adequate despite the adiposity levels in the group with Achondroplasia, as a linear relationship is observed in agonist and antagonist EMG between groups of differing adiposity (De Vito et al., 2003). Boundaries of the BF were determined using ultrasonography (Technos MXP Biosound Esaote) to ensure consistent placement of EMG electrodes over the KF. When established two pre-gelled, unipolar, 10mm, Ag-AgCl percutaneous electromyography (EMG) electrodes (Ambu Neuroline 720, Baltorpbakken, Denmark) were placed distally at ~1/3 of muscle length, to avoid the motor unit of the BF, and ~2 mm apart along the mid-sagittal plane of the muscle (NORAXON, Arizona, USA). A third electrode was placed on the lateral epicondyle of the same femur as a reference (see Figure 5.1 in Chapter 5 for an example of the experimental set up). Prior to the placement of the electrodes, areas of the skin were shaved, then cleaned using an alcoholic wipe to minimise skin impedance and hence improve the EMG signal. Raw EMG data were recorded at 2000 Hz, with a high and low band-pass filter set at 10 and 500 Hz respectively, and a notch set at 50 Hz. The integral of the root mean square was recorded 0.5 seconds either side of the KE and KF iMVCτ to quantify the level of KF muscle coactivation. Based on a linear relationship occurring between torque and EMG activity (Maganaris et al., 1998), KF

torque during KE iMVC was derived by converting the percentage activation of KF EMG during KE iMVC to KF EMG during KF iMVC.

$$Equation\ 6.3: KF\tau = \left(\frac{\left((KE \div KF) \cdot 100\right)}{100}\right) \cdot KF\ iMVC\,\tau$$

Where KFτ is the KF torque during KE (N·m), KE is the agonist EMG (mV) recorded of the KE during KE iMVC, KF is the antagonist EMG (mV) recorded of the KE during KE iMVC and KF iMVCτ is the torque (N·m) observed during KF iMVC.

The measurement of agonist and antagonist muscle activation are required for the accurate quantification of net KE iMVCτ production, both of which are used in the calculation of specific force (Maganaris et al., 1998; Stebbings et al., 2014) . Therefore, net KE iMVCτ was given as the sum of KE iMVCτ and KFτ while a ratio of KF iMVCτ and KE iMVCτ was calculated to describe a balance of quadriceps to hamstring strength.

6.3.8 Moment arm

A dual energy X-ray absorptiometry (DEXA) scanner (Hologic Discovery, Vertec Scientific Ltd, UK) was used to obtain moment arm length of the patellar tendon at an knee flexion angle of 90° (Erskine et al., 2014). These methods are described in section 5.3.2.7 and also presented as an experimental set up in Figure 5.4 of Chapter 5.

6.3.8 Patella tendon force

F_{PT} was calculated by dividing net KE iMVC torque by the patella moment arm length (m).

6.3.9 Stress and strain

Tendon strain was the ratio of excursion to resting patella tendon length (%) while tendon stress was calculated by dividing F_{PT} by CSA_{PT} at mid-tendon length (MPa) (Maganaris and Paul, 2000b; Reeves et al., 2003a; Maganaris et al., 2006; Onambélé et al., 2007; Seynnes et al., 2009; O'Brien et al., 2010b).

6.3.10 Tendon stiffness

Patella tendon force-elongation relationships were fitted with second order polynomial functions forced through zero. Instantaneous patella tendon stiffness values were then calculated at 10% intervals of KE iMVC force (from 10-100%), from the gradient of tangential lines along the force-elongation curve (Onambélé et al., 2007).

6.3.11 Young's modulus

Instantaneous Young's modulus values were calculated as:

$$Equation\ 6.4: Young's\ Modulus\ =\ K\ \times \left(\frac{L_{PTi}}{CSA_{PT}}\right)$$

Where *K* is the calculated stiffness, L_{PTi} is the patella tendon length at each 10% tangential calculation of F_{PT} and CSA_{PT} is the cross-sectional area of the patella tendon at 50% of resting length.

6.3.12 Standardised measures of tensile properties

With large discrepancies in maximal F_{PT} expected between groups (Chapter 5), the tensile properties of the patella tendon were calculated at the lowest F_{PT} attained by the weakest participant with Achondroplasia. Patella stress, strain and Young's Modulus are then presented at this common force level (1756 N) achieved by all participants consistent with previous work (Onambélé et al., 2007; Burgess et al., 2009; Hicks et al., 2017).

6.3.12 Statistical analysis

All data were collated onto a personal computer (MacBook Pro, California) and analysed using SPSS (v22.0, IBM). Data were assumed to be parametric following Shapiro-Wilk and Levene's tests. Repeated measures ANOVA with between group effects was conducted on the CSA_{PT} and VL architecture. Between group comparisons for all measured variables at 10% intervals of iMVC were conducted using independent t-tests. Intraclass correlations (ICC) with a one-way random effects model and CV was used for reliability of KE iMVCτ between proximal and distal effort and between post scan digitisation of CSA_{PT} and patella tendon elongation. Where data violated parametric assumptions, a Mann Whitney-U was performed. Alpha was set at ≤ 0.05 while all results are reported as means (SD).

6.4 Results

6.4.1 Anthropometric measures and reliability measures

The description of participants' anthropometrics are given in Table 2.1 of Chapter 2. Reliability of KE iMVCτ between proximal and distal efforts were strong (group with Achondroplasia: ICC = 0.968, CV = 3.2%, P < 0.001; controls: ICC = 0.932, CV = 3.4%, P < 0.001) as were the measures of CSA_{PT} (ICC = 0.965 – 0.998, CV 1.3 – 4.4% for all measures in both groups, P < 0.001) and patella tendon elongation (ICC = 0.940 – 0.982, CV 1.5 – 3.0% for all measures in both groups, P < 0.001).

6.4.2 Architectural properties of the patella tendon and vastus lateralis at rest

The group with Achondroplasia had a 32% smaller resting patella tendon length (P < 0.001) and a 41% smaller VL than controls (P < 0.001, Table 6.1). ANOVA showed an effect in CSA_{PT} between groups (P = 0.004), but no interaction effect was found (P = 0.868), nor any effect in CSA_{PT} between each measured interval within groups (P = 0.051). The group with Achondroplasia had a smaller CSA_{PT} at 25% (P = 0.013), 50% (P = 0.003) and 75% (P = 0.010) compared to controls (Table 6.1). Patella tendon volume was 48% less in the group with Achondroplasia compared to controls (P < 0.001). There was no difference between groups' moment arm length (P = 0.989, Table 6.1). The group with Achondroplasia did have a 15% longer patella tendon length relative to VL length (P = 0.001) and a 42% greater moment arm length to femur ratio, compared to controls (P < 0.001, Table 6.1). Whereas there was no difference in the ratio of 50% CSA_{PT} to patella tendon length between groups (P = 0.102, Table 6.1).

6.4.3 KE and KF iMVCτ

The group with Achondroplasia produced 63% less KE iMVCτ than controls ($P < 0.001$, Table 6.2). KF iMVCτ was 82% lower in the group with Achondroplasia compared to controls ($P < 0.001$, Table 6.2). The ratio of KE to KF iMVCτ was significantly higher in the group with Achondroplasia compared to controls ($P < 0.001$, Table 6.2). The ratio between KE iMVCτ and VL length was 39% greater in the group with Achondroplasia compared to controls ($P < 0.001$, Table 6.2).

Table 6.1: Morphological properties of the patella tendon, vastus lateralis and patella tendon moment arm during rest in adults with Achondroplasia and control. Values displayed as mean (SD).

	Achondroplasia		Control
PT Length (mm)	37.6 (4.3)	*	55.2 (5.8)
VL Length (cm)	19.8 (1.2)	*	33.6 (1.7)
PT Length:VL length (%)	19.1 (2.3)	*	16.2 (1.7)
25% CSA_{PT} (mm^2)	81.6 (8.7)	†	104.1 (25.4)
50% CSA_{PT} (mm^2)	86.9 (13.8)	‡	110.3 (19.6)
75% CSA_{PT} (mm^2)	79.8 (15.5)	†	105.1 (25.7)
50% CSA_{PT}:PT Length (%)	0.45 (0.11)		0.51 (0.08)
PT Volume (mm^3)	82.8 (11.4)	*	106.5 (22.2)
VL Fascicle Length:VL Length (%)	0.48 (0.13)	*	0.31 (0.04)
Moment Arm (mm)	37.6 (4.3)		37.6 (2.1)
Moment arm:Femur length (%)	19.1 (2.8)	*	11.1 (0.8)

PT: Patella Tendon; VL, Vastus Lateralis; CSA_{PT}: patella tendon cross sectional area. † $P < 0.05$, ‡ $P < 0.01$, * $P \leq 0.001$.

Table 6.2: Activation and force characteristics of the adult VL in adults with Achondroplasia and controls. Values presented as mean (SD).

	Achondroplasia		Control
KE iMVCτ (N·m)	92.8 (5)	*	260.1 (9.5)
KF iMVCτ (N·m)	19.0 (7.2)	*	105.0 (19.2)
KE iMVCτ:VL Length	0.20 (0.04)	*	0.13 (0.02)
Activation (%) †	83.9 (13.9)		92 (5.9)
Coactivation (%) †	42.6 (20)	*	12.6 (5.3)
Net KE iMVCτ (N·m)	100.1 (21.7)	*	273.7 (37.9)

KE, knee extensors; KF, knee flexors; iMVCτ, isometric maximal voluntary contraction torque; VL, vastus lateralis; † Mann Whitney-U; * $P \leq 0.001$.

6.4.4 Activation and coactivation

There was no difference in maximal KE activation between the groups (P = 0.125), but the group with Achondroplasia had 70% greater coactivation of the BF during KE iMVC compared to controls (P < 0.001, Table 6.2).

6.4.5 Net iMVCτ

Both groups increased KE iMVCτ by ~6% in both groups when corrected for BF coactivation (P < 0.001). The net KE iMVCτ produced by the VL was 63% less in the group with Achondroplasia compared to controls (P < 0.001, Table 6.2).

6.4.6 Muscle contractile properties

There was no difference in VL pennation angle (P = 0.105) or fascicle length (P = 0.199) at rest between groups. From rest to iMVC, the fascicles of the group with Achondroplasia shortened 28% more (P = 0.012) and increased in pennation angle by 25% compared to controls (P = 0.029, Figure 6.2 and Figure 6.5). The group with Achondroplasia had a 36% longer fascicle length relative to VL length than controls (P < 0.001, Table 6.1).

6.4.7 Patella tendon force-elongation relationship

The group with Achondroplasia produced 64.3% less maximal F_{PT} than controls (P < 0.001) and an average of 63.4% less F_{PT} at each 10% interval (Figure 6.6a and Table 6.3). Tendon elongation was 21.5% less at KE iMVC in the group with Achondroplasia

compared to controls (P < 0.001) with an average 15% less elongation at each 10% interval (each 10% interval: P < 0.01, Figure 6.6a and Table 6.3).

6.4.8 Tendon stress-strain

Patella tendon strain was similar in the group with Achondroplasia and controls at KE iMVC and at each 10% interval, with maximal strain being 13.0 (4.1%) and 12.6 (3.3%) for the groups, respectively (P > 0.05, Figure 6.6b and Table 3). However, for each corresponding interval of strain, stress was on average 54.5% lower in the group with Achondroplasia at each 10% interval compared to controls (each 10% interval: P < 0.001) with the maximal stress being 52.6% lower (P < 0.001, Figure 6.6b and Table 6.3).

6.4.9 Tendon stiffness

Patella tendon stiffness was on average 51.1% lower through the 10-90% intervals of KE iMVC in the group with Achondroplasia compared to controls (each 10% interval: P < 0.01). The maximal patella tendon stiffness of the group with Achondroplasia was 47.3% more compliant than controls (P < 0.001, Table 6.3).

6.4.10 Young's modulus

Young's Modulus was on average 53.8% lower in the group with Achondroplasia at each 10% of KE iMVC compared to controls (each 10% interval: P < 0.001, Table 6.3

and Figure 6.6c) and was 50.7% lower at KE iMVC ($P < 0.001$, Table 6.3 and Figure 6.6c).

Table 6.3: Patella tendon properties at 10% intervals for the group with Achondroplasia and controls. Values displayed as mean (SD).

	Torque (N·m)		Tendon Length (mm)		Force (N)		Strain (%)		Stress (MPa)		Stiffness (N·mm^{-1})		Youngs Modulus (GPa)	
% Max	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon	Control	Achon
10	25.4 (3.8) *	8.9 (1.9)	52.4 (4.3) *	38.3 (3.9)	677 (99) *	241 (62)	2.7 (1.5)	3.3 (2.3)	6.3 (1.4) *	2.8 (0.8)	668 (19) *	271 (24)	0.32 (0.05) *	0.12 (0.03)
20	50.8 (7.5) *	17.8 (3.9)	53.2 (4.8) *	39.1 (3.8)	1353 (197) *	482 (125)	4.0 (2.5)	5.2 (3.5)	12.6 (2.8) *	5.6 (1.5)	786 (32) *	354 (37)	0.39 (0.07) *	0.17 (0.04)
30	76.2 (11.3) *	26.7 (5.8)	54.1 (4.6) *	39.5 (3.5)	2030 (296) *	723 (187)	5.6 (2.8)	6.3 (3.2)	18.9 (4.3) *	8.5 (2.3)	888 (43) *	420 (47)	0.45 (0.08) *	0.20 (0.05)
40	101.6 (15.1) *	35.6 (7.7)	54.8 (4.8) *	40.0 (3.6)	2707 (394) *	964 (250)	6.8 (3.0)	7.4 (3.2)	25.2 (5.7) *	11.3 (3.0)	979 (51) *	478 (55)	0.50 (0.09) *	0.23 (0.06)
50	127.0 (18.9) *	44.6 (9.7)	55.4 (4.8) *	40.5 (3.4)	3384 (493) *	1205 (312)	7.9 (3.0)	8.5 (3.7)	31.5 (7.1) *	14.1 (3.8)	1063 (59) *	529 (63)	0.55 (0.10) *	0.26 (0.06)
60	152.4 (22.6) *	53.5 (11.6)	55.8 (4.9) *	41.0 (3.5)	4060 (591) *	1446 (375)	8.5 (3.3)	9.7 (3.4)	37.9 (8.5) *	16.9 (4.5)	1141 (66) *	576 (69)	0.59 (0.10) *	0.28 (0.07)
70	177.8 (26.4) *	62.4 (13.5)	56.1 (5.0) *	41.6 (3.1)	4737 (690) *	1687 (437)	9.0 (3.2)	11.0 (4.1)	44.2 (9.9) *	19.7 (5.3)	1213 (73) *	619 (75)	0.63 (0.11) *	0.31 (0.07)
80	203.2 (30.2) *	71.3 (15.4)	56.7 (5.1) *	41.9 (3.3)	5414 (789) *	1928 (500)	9.9 (3.3)	11.7 (3.9)	50.5 (11.4) *	22.5 (6.0)	1281 (79) *	659 (80)	0.67 (0.12) *	0.33 (0.08)
90	227.9 (34.6) *	80.2 (17.4)	57.4 (5.0) *	42.1 (3.5)	6071 (904) *	2169 (562)	11.0 (3.4)	12.0 (3.6)	56.6 (12.7) *	25.4 (6.8)	1345 (86) *	697 (86)	0.72 (0.12) *	0.35 (0.08)
100	260.1 (37.9) *	92.8 (20.0)	58.4 (4.9) *	42.5 (3.4)	6931 (1005) *	2511 (659)	12.6 (3.3)	13.0 (4.1)	64.5 (14.0) *	29.4 (8.0)	1418 (101) *	748 (93)	0.77 (0.14) *	0.38 (0.09)

Achon: Achondroplasia. Comparisons made between Achondroplasia and control at each percentile only. * $P \leq 0.001$.

6.4.11 Standardised tendon properties

With such large discrepancies in maximal F_{PT} between groups being observed, the lowest F_{PT} attained by the weakest participant with Achondroplasia (1765 N) was used as a comparison between the two groups (Table 6.4). As was with maximal and 10% intervals of F_{PT}, similar differences persisted when standardised. Stress (21.5%, P = 0.001) and strain (77.7%, P < 0.001) were higher in the group with Achondroplasia, while stiffness (11.7%, P < 0.001) and Young's Modulus (11.1%, P = 0.041) were lower compared to controls (Table 6.4).

Table 6.4: Elastic properties of the patella tendon at a standardised force (1756 N) in adults with Achondroplasia and controls. Values displayed as mean (SD).

	Achondroplasia		Control
Stress (MPa)	20.9 (3.2)	*	16.4 (2.8)
Strain (%)	11.2 (3.8)	*	2.5 (2.0)
Stiffness ($N \cdot mm^{-1}$)	637 (39)	*	721 (10)
Young's Modulus (GPa)	0.32 (0.06)	*	0.36 (0.06)

* $P \leq 0.001$.

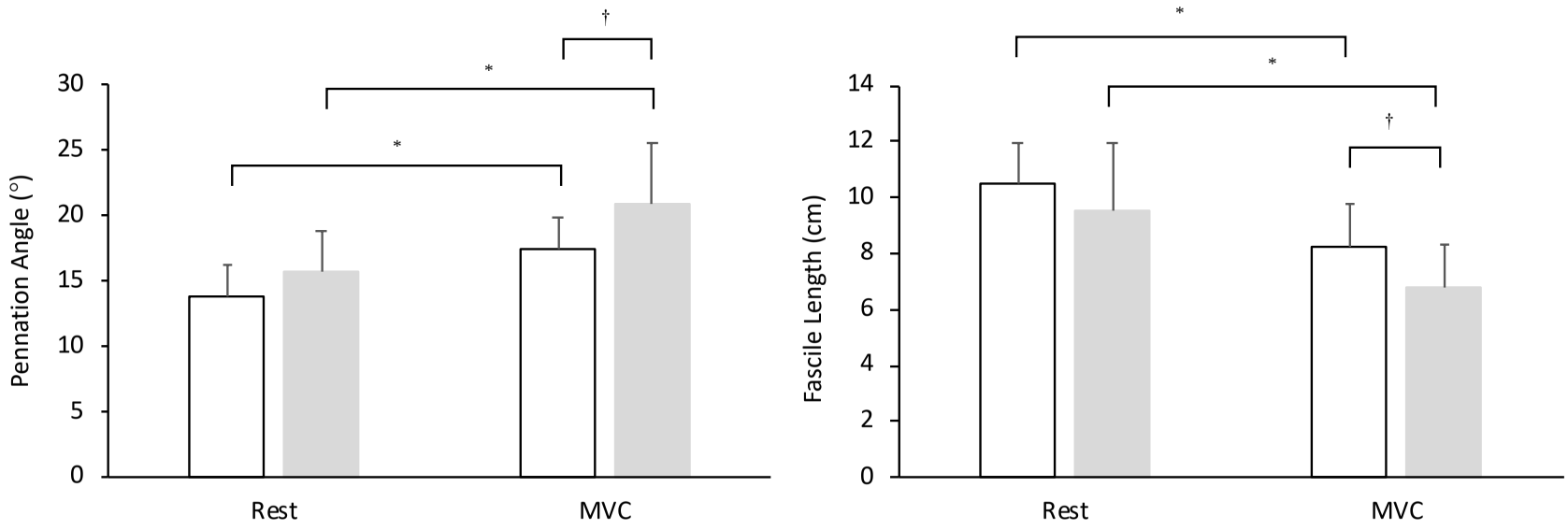

Figure 6.5: Architectural properties of the vastus lateralis' pennation angle (left) and fascicle length (right) in adults with Achondroplasia (grey) and controls (white) from rest to iMVC. $^{*} \leq 0.05$ $^{\dagger} \leq 0.001$.

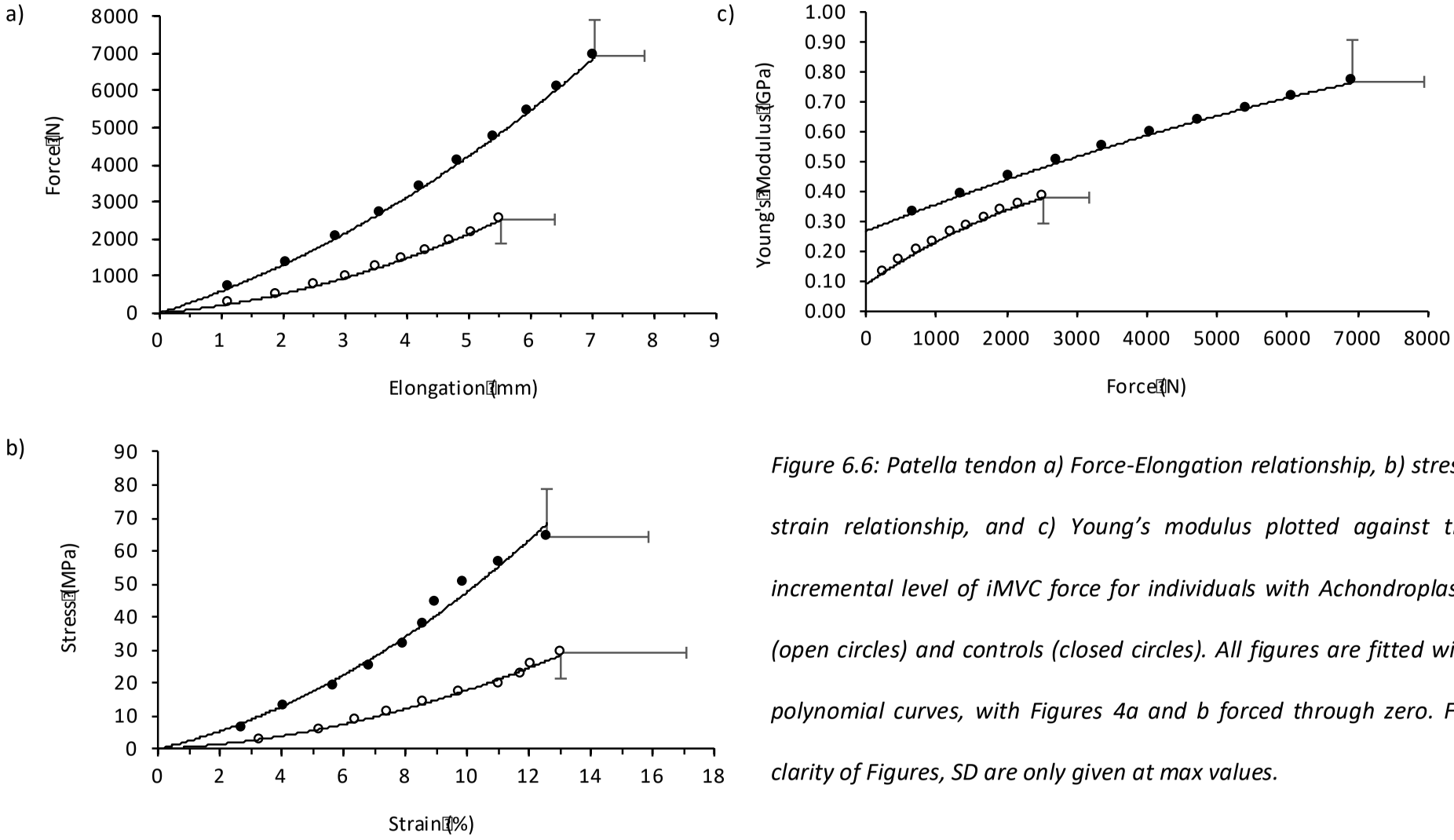

Figure 6.6: Patella tendon a) Force-Elongation relationship, b) stress-strain relationship, and c) Young's modulus plotted against the incremental level of iMVC force for individuals with Achondroplasia (open circles) and controls (closed circles). All figures are fitted with polynomial curves, with Figures 4a and b forced through zero. For clarity of Figures, SD are only given at max values.

6.5 Discussion

The aim of this study was to measure and compared the *in vivo* morphological and material properties of patella tendon during KE iMVC in adults with Achondroplasia and controls. The patella tendons of individuals with Achondroplasia was smaller in CSA and volume but were disproportionate in length compared to controls. The main finding however, was that despite accounting for patella tendon morphology and mechanical properties, the Young's Modulus of the patella tendon was lower in the group with Achondroplasia than controls' leading to a more compliant patella tendon.

6.5.1 Patella tendon morphology, force, strain, stress and stiffness

The morphological properties of the patella tendon from the present control group were similar to those previously observed (Onambélé et al., 2007; Seynnes et al., 2009; O'Brien et al., 2010b; O'Brien et al., 2010d). Many agree that an increased ratio of tendon CSA to tendon length increases tendon stiffness (Kubo et al., 2001b; Reeves, 2006; O'Brien et al., 2010b). While this ratio was higher in the patella tendon of the group with Achondroplasia compared to controls, a higher tendon stiffness was not observed. A shorter femur length of the individuals with Achondroplasia compared to controls (measured here as VL length) would explain the difference in groups' patella tendon length. A shorter femur would suggest a shorter muscle tendon unit, which is observed elsewhere in groups of shorter statures (Morse et al., 2008; O'Brien et al., 2010b; O'Brien et al., 2010d). However, the shorter femur observed in the group with Achondroplasia does not explain the their relatively

longer patella tendon length compared to controls. In individuals with Achondroplasia, more flexion of the knee is observed when at rest compared to controls (Akyol et al., 2015). This more flexed joint position may deform the patella tendon over time leading to a creep effect. However, no longitudinal measures of the group's patella tendon length have been made.

With no *in vitro* tendon properties identified in any population with Achondroplasia, explanations as to why their absolute measures of CSA_{PT} are lower and relative measures (CSA_{PT} to patella tendon length) are higher compared to controls are speculative. The measurement of CSA_{PT} using ultrasound is regarded by some, though very few, as unreliable (Ekizos et al., 2013). However, previous reports, and in this Chapter, show high ICCs for CSA_{PT} with a typical error of <1.5 mm^2 (Reeves et al., 2003a; Gellhorn and Carlson, 2013). Using the maximal CSA_{PT} difference from Reeves et al., the ranges of calculated stress measured here would be 3.6 and 2.8% different for the group with Achondroplasia and controls, respectively. Even the lower ICCs presented by Ekizos et al. would present stress values ±20% of the average values for the groups included in this Chapter. If the measurement of CSA_{PT} was indeed miscalculated in this Chapter, it would have to have been ~40% under- and over-predicted for the group with Achondroplasia and controls, respectively, for there to be the same stress and therefore the same stiffness values.

The use of ultrasound is a good method of attaining CSA_{PT} without the use and availability of a magnetic resonance imaging (MRI) scanner, which is considerably less accessible for most researchers. Despite the conflicting arguments in the reliability

of CSA_{PT} calculation, the patella tendon stress and Young's Modulus calculated here have a large amount of face validity. These differences in patella tendon stress and Young's Modulus between the observed groups are undoubtedly due to the large differences in relative (CSA_{PT} to patella tendon length) and absolute CSA_{PT} between the groups. Importantly though, this is backed by the potential respective under- and over-predictions of the group with Achondroplasia and controls' CSA_{PT} required to attain similar stress values. Therefore, the smaller absolute CSA_{PT} seen in the group with Achondroplasia would most likely be a result of the scaling of leg length and muscle size. The higher relative CSA_{PT} in the group with Achondroplasia is most probably 'pseudohypertrophy' of the tendon fibrils and is likely to be due to intrinsic factors of the tendon, which are discussed later.

The values of patella tendon stress in the control group were similar to those measured previously (Maganaris, 2002; Onambélé et al., 2007) with values approaching the maximal tensile strength of human patella tendons (0.65 GPa, (Johnson et al., 1994). The group with Achondroplasia produced 53% less stress at iMVC than controls with no difference in agonist activation was observed between groups (84 and 92% for Achondroplasia and controls respectively). This suggests that the groups' strain and stress values were close to their maximal. With similar *in vivo* values of maximal strain being observed in other groups (Maganaris, 2002; Onambélé et al., 2007), it would be assumed that the rupture point of the patella tendon would occur at a reduced absolute force in individuals with Achondroplasia compared to controls. Indeed, the lower Young's Modulus of individuals with Achondroplasia infers a weaker patella tendon at all 10% increments of iMVC, but

importantly at a commonly attained force value (1756 N). Therefore, the more compliant and probably lower tensile strength in of the patella tendon in individuals with Achondroplasia is likely due to differences in intrinsic properties of the tendon.

6.5.2 Young's modulus

Given the size of the difference in the patella tendon's Young's Modulus (here defined as tendon compliance) between individuals with Achondroplasia and controls found in this Chapter, there appears to be no conclusive evidence to suggest why such a large difference exists. There are however a number of speculative but reasonable explanations as to why the patella tendons if individuals with Achondroplasia would be more compliant. Whilst this list is not exhaustive, the mutation which causes Achondroplasia (FGFR3), the tendon extracellular matrix (ECM), physical activity levels, hormonal differences and body morphology of the group may all contribute to their more compliant patella tendon.

The most likely contributor to the more compliant patella tendon in individuals with Achondroplasia is the mutation of the FGFR3, which is linked unequivocally to bone plate formation and growth (Deng et al., 1996; Horton et al., 2007; Superti-Furga and Unger, 2007; Krakow and Rimoin, 2010). FGFR3 is part of a family of fibroblasts which are all essential for the development, repair and turnover of collagen (Benjamin and Ralphs, 2000). In FGFR3 mutated mice, Type II collagen in the fibrocartilage is shown to be negatively affected (Liang et al., 2009). Fibrocartilage extends from bone to tendon (Benjamin et al., 2002) and if the assumption that fibrocartilage is similarly

affected by FGFR3 in humans, the compliance of the fibrocartilage may be reduced. However, with the human tendon predominantly made from Type I and III collagen, any evidence that the presented theory exists would not account for the large difference in patella tendon compliance between groups observed in this Chapter. Despite this, the evidence found in FGFR3 mutated mice may show that other collagen types may in turn be affected in the tendon. Whilst this is speculative, the FGFR3 is a collagen affecting mutation and with there being no *in vitro* data available from the tendons of individuals with Achondroplasia, any evidence contradicting this theory is yet to be presented.

Assuming that FGFR3 does not substantially affect the tendon growth or formation in individuals with Achondroplasia (if at all), the way in which the ECM of the collagen fibres are arranged within the tendon would be a likely cause of their more compliant patella tendon compared to controls. Studies have shown that a more compact orientation of the tendon ECM aids in the transmitted of force from the muscle to bone (Lieber et al., 2003). The way in which the ECM is orientated is dependent on the signalling pathways, which are activated by force development and activation of the muscle-tendon unit (J. H. Wang, 2006). The group with Achondroplasia included here were self-reported active adults who regularly partook in sporting activities such as badminton and basketball (Section 2.3 of Chapter 2). Not only are ground reaction forces higher in these activities compared to general physical activity, such as walking, individuals with Achondroplasia have shorter legs than controls (Chapter 2). Shorter legs increase stride frequency compared to longer legs when walking and running at set speeds (Vaughan and O'Malley, 2005). Furthermore, due to the

disproportionate limb length of individuals with Achondroplasia, their body mass is also disproportionate compared to controls; shown in this thesis (Chapter 2) and by others' measures of BMI (Horton et al., 1978a; Hecht et al., 1988; Owen et al., 1990; Hoover-Fong et al., 2007). A greater upper-body mass combined with a shorter stride length and increased stride frequency, during activities such as walking and running, will lead to a higher ground reaction force and therefore higher muscle activation during contraction of the lower limb muscles compared to controls. These factors are likely to stimulate cell signalling of pathways of the ECM in individuals with Achondroplasia, but there are no *in vitro* data in the population's tendons to support this suggestion.

It has been shown that muscle fibres require force whilst under strain to stimulate adaptation (McMahon et al., 2013; McMahon et al., 2014), while tendons can require as little as 4% strain to achieve microscopic failure and further adaptation (Butler et al., 1978). For the group with Achondroplasia, during activities such as running, it may be that the quadriceps are not under sufficient strain during the loading phase of gait for the patella tendon to be at a threshold by which the appropriate cell signalling occurs which in turn leads to tendon adaptation, but this is yet to be observed empirically. Some gait analysis has been conducted in groups with Achondroplasia (Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008), but little data are available in adults with the condition. In addition, no kinetic analysis of force development or *in vivo* tendon excursion during gait is available in individuals with Achondroplasia which would have helped to explain some of the findings observed here.

The water content within the human tendon is reported to be between 55-70% and is used for spacing of fibrils and lubricant (Kjær 2004). Laaksonen et al. (2003) describes obese people having greater total-body water content compared to lean individuals. Chapter 2 identified the group with Achondroplasia as having higher adipose fat content compared to controls, a find backed by many (Horton et al., 1978a; Hecht et al., 1988; Owen et al., 1990; Hoover-Fong et al., 2007). A greater water content in the body is therefore likely to be observed in individuals with Achondroplasia. In animal models, a higher water content of the body changes the orientation of the ECM and increases tendon compliance (Birch, 2007). Here, the possible higher water content of individuals with Achondroplasia may be observed in the patella tendon; here as a pseudohypertrophy of CSA_{PT}. Furthermore, lower levels of oestrogen receptor-α are linked to a higher fat mass in mice (Lindberg et al., 2001). A higher availability of oestrogen inhibits the growth and turnover of collagen fibres in females (Henneman, 1968; B. F. Miller et al., 2007; Hansen et al., 2009), leading to a more compliant tendon in the same population (Kubo et al., 2001b; Onambélé et al., 2007). Similar levels of oestrogen are observed in obese males to that of lean females (Gates et al., 2012). Combining the findings of higher adiposity in individuals with Achondroplasia (Chapter 2) with the aforementioned findings in bodies with higher fat content, it could be suggested that individuals with Achondroplasia do indeed have greater oestrogen levels, which are hindering collagen turnover and negatively affecting tendon stiffness. Both water content and oestrogen levels are speculative in the current group with Achondroplasia though. More work involving the intrinsic properties of the patella tendon using *in vivo* and *in vitro* methods is needed in this group to support the theories presented above.

6.5.3 Biomechanical implications

To the author's knowledge, the data presented in this Chapter is the first to identify the *in vivo* fascicle shortening of muscles during contraction in individuals with Achondroplasia (Figures 6.2 and 6.5). Results show that fascicle shorten more while pennation angle increases more in individuals with Achondroplasia compared to controls. This finding is not only novel, but also is partially explained by the patella tendon compliance of the group. Where a more compliant tendon exists, a left-ward shift in the length tension relationship is observed in the muscle fascicles (Reeves, 2006). This results in more fascicle shortening and increased pennation angle during muscle contraction, in turn lowering effective force production. To account for the latter, either muscles would have to activate more to achieve the required force level to complete a submaximal task, or a structure may damage as a result of an inactive muscle. At submaximal tasks, individuals with Achondroplasia are likely to have differences in postural balance greater risk of tendon compared to controls. Both of which are observed in other populations with compliant tendons (Onambélé et al., 2006; Onambélé et al., 2008).

The results of this Chapter suggest that individuals with Achondroplasia transfer of force from muscle to bone less effectively that controls. This would have implications for cyclic activities such as walking and running, due to a likely increased hysteresis loop in the tendon (Maganaris and Paul, 2000a). It has been shown that stride frequency is greater in children with Achondroplasia at self-selected walking speeds (Inan et al., 2006). Assuming this finding is consistent within adults with Achondroplasia, more muscle activation per stride would be required to maintain a

set speed compared to controls, which would incur a higher energetic cost (Saunders et al., 2004; Arampatzis et al., 2006; Fletcher et al., 2010), which is observed in Chapter 4. While the higher tendon compliance of individuals with Achondroplasia may play a role in their higher energetic cost of walking and running compared to controls, it is likely that this only accounts for a small proportion of the overall difference in energy cost.

The combination of a higher relative mass of the torso and lower relative mass of the legs (Chapter 2), lower relative force production (Chapter 5) and higher tendon compliance (current Chapter) in adults with Achondroplasia is likely to affect the general gait pattern compared to controls. This in turn is likely to have a negative impact on the energy expenditure during gait of individuals with Achondroplasia (i.e. a higher metabolic cost). While there are kinematic data available to describe the gait of young individuals and leg lengthened adults with Achondroplasia (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008), there does not appear to be a detailed kinematic analysis of gait in adults with Achondroplasia.

6.6 Conclusion

This study aimed to examine the *in vivo* material properties of the patella tendon during a ramped MVC in adults with Achondroplasia and controls. The main finding is that individuals with Achondroplasia have a more compliant patella tendon than controls. In addition, at both absolute (iMVC) and standardised force (1756 N) levels

of stress, stiffness and Young's modulus are lower in the group with Achondroplasia compared to controls suggesting intrinsic differences between groups.

Chapter 7: The kinematic analysis of walking and running in adults with Achondroplasia

Part 1: Introduction and methods

Part 2: Walking results

Part 3: Running results

Part 4: General kinematic discussion

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7.1 Abstract

This Chapter aimed to describe the spatio-temporal, discrete joint kinematics and centre of mass (CoM) kinematics of gait during a number of incremental walking and running speeds in individuals with Achondroplasia and compare them to age-matched controls. Three-dimensional motion analysis (VICON, Plug-in-gait) of gait was conducted over ground at a self-selected walking speed (Achondroplasia 1.02 (0.13) $m \cdot s^{-1}$; controls 1.44 (0.14) $m \cdot s^{-1}$), 6-fixed walking (0.56 - 1.94 $m \cdot s^{-1}$, step 0.28 $m \cdot s^{-1}$) and 6-fixed running speeds (1.67 – 3.33 $m \cdot s^{-1}$, step 0.28 $m \cdot s^{-1}$). Spatio-temporal measures of one stride (heel contact-to-heel contact) and discrete joint kinematics for the pelvis, hip, knee and ankle in all three anatomical planes were presented in, and compared between, both groups. The Gait Profile Score (GPS) was the calculated from 15 related gait kinematics to calculate a global joint kinematic score for the gait of individuals with Achondroplasia to be compared to controls. Local minima and maxima of the vertical and medio-lateral CoM translation were presented absolutely, relative to stature and relative to the height of its position at heel contact for both groups. Two-way ANOVAs with between effects were used to determine differences between groups' gait kinematics. The group with Achondroplasia had a shorter stride length and higher frequency than controls at all walking and running speeds. There were numerous differences in discrete joint kinematics between groups, which led to the group with Achondroplasia presenting as more 'flexed' than controls during walking and running. The GPS of the group with Achondroplasia was higher than the controls at all walking speeds, but differences lessened between groups when running. The group with Achondroplasia had less relative vertical translation of the

CoM, but more medio lateral translation than the control group during walking and running. The data from this Chapter suggest that gait of individuals with Achondroplasia is quantifiably different to controls. The difference in gait between groups may exist due to the body dimensions of the group with Achondroplasia requiring a more flexed position to void fall incidence.

Part 1: Introduction and General Methods

7.2.p1 Introduction

The previous Chapters in this thesis have described and compared total-body and segmental anthropometry, maximal oxygen consumption ($\dot{V}O_{2max}$), submaximal oxygen consumption ($\dot{V}O_2$) during incremental exercise and musculotendon properties during maximal voluntary contraction in adult males with Achondroplasia to age matched average statured males (controls). One of the main findings thus far is that, despite incorporating body morphology and leg length, the metabolic cost (C) of adults with Achondroplasia during walking and running is higher compared to controls (i.e. more $\dot{V}O_2$ is required for a given distance, Chapter 4). Chapter 5 and 6 identified that adults with Achondroplasia are both weaker and have a more compliant patella tendon than controls. These are both likely to contribute to the difference between groups' C during gait (Saunders et al., 2004; Arampatzis et al., 2006; Fletcher et al., 2010). It is probable that some of the difference in C between groups is also accounted for by an altered limb and centre of mass (CoM) movement during walking and running between groups. To the author's knowledge though, kinematics of the CoM during gait in individuals with Achondroplasia has not been measured.

Self-selected walking (SSW) appears coordinated and efficient in the healthy individual (Umberger and Martin, 2007), whereas in groups where musculoskeletal pathology exits, gait can be anecdotally and empirically different to controls (Baker et al., 2009). In such groups, differences in gait kinematics are explained by neurological impairment, muscle weakness, amputation or skeletal deformity (van

den Hecke et al., 2007; Baker et al., 2009; Beynon et al., 2010; Baker et al., 2012; Zollinger et al., 2016; Weinert-Aplin et al., 2017). While individuals with Achondroplasia appear to be unaffected by such pathologies, their shorter legs (Chapter 2), differences in joint morphology (Akyol et al., 2015) and greater relative torso mass compared to controls is likely to affect their gait kinematics. To date there appear to be four data sets that describe kinematic variables of the pelvis, hip, knee and ankle of SSW gait in individuals with Achondroplasia, all of which show differences in joint kinematic patterns compared to controls (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008). However, the groups with Achondroplasia included in these studies do not allow for an accurate gait description of adults with Achondroplasia who have not undergone limb lengthening surgery. Furthermore, due to the difference in body size and dimensions between groups, it would be appropriate to normalise gait parameters, through such methods as non-dimensional normalisation (NDN) (Hof, 1996). Including such measures would help explain the movement patterns of individuals with Achondroplasia further.

With the large number of kinematic variables that are collected during gait, quantifying whether a person, or population, is different to another is difficult. Methods have been developed to describe a global gait score for clinical populations to enable comparison to control populations by incorporating a number of different kinematic variables. One such method is the Gait Profile Score (GPS); a lower GPS values represent a more comparable movement pattern to that of a control population over a complete stride (Baker et al., 2009). GPS has been used to compare

differences in gait between individuals with and without gait pathologies and has been conducted under various conditions (Baker et al., 2009; Beynon et al., 2010; Baker et al., 2012; Kark et al., 2012; Johansson et al., 2014; Schweizer et al., 2014). It is derived by summing 15 root mean square (RMS) differences in gait related kinematics during the gait cycle. GPS correlates well with clinical assessments (Baker et al., 2012), has high face validity (Beynon et al., 2010) and affords the ability to perform more powerful statistical analyses than other global gait difference scores, such as the Gait Deviation Index (Schwartz and Rozumalski, 2008). Calculation of GPS allows for inter- and intra-joint and plane comparisons within and between groups with different pathologies. This is useful to determine which are the predominant joints affecting gait differences and therefore aid in gait rehabilitation or gait improvement interventions. Descriptions of gait in populations with Achondroplasia are sparse, but do show individual joint differences compared to controls (Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008); these though are not inferentially compared as can be done using GPS.

Accurate assessment of joint kinematics in turn allows for an estimation of the body's CoM position, which is useful during gait analysis as it can allude to the mechanical work during locomotion (Cavagna and Kaneko, 1977). The amplitude of the CoM's vertical movement is determined, somewhat, by leg length and so for individuals with Achondroplasia, the position of the CoM is likely to be lower than controls. While it may be closer to the floor, the vertical movement and displacements patterns are at present unknown. Such measures though would help with explaining some of the difference in C observed between groups in Chapter 4.

The aim of this chapter was to provide a kinematic analysis of gait in non-leg lengthened individuals with Achondroplasia over a range of speeds. The specific objectives of this chapter are to:

1) analyse the gait of individuals with Achondroplasia over a range of walking speeds that match those used in Chapter 4 during submaximal $\dot{V}O_2$ analysis;
2) present spatial and temporal gait kinematics and calculate GPS during walking and running in individuals with Achondroplasia;
3) describe vertical CoM movements during walking and running in individuals with Achondroplasia;
4) compare all variables to controls.

7.3.p1 Method

7.3.p1.1 Participants

Ten adults with Achondroplasia and 17 age matched controls that were free from lower limb injury volunteered to participate in the study and are described in Table 2.1 in Chapter 2.

7.3.p1.2 Biomechanical measures

Three-dimensional motion analysis hardware (VICON Motion Systems, Oxford, UK, 100 Hz) was used to determine gait parameters during SSW. The Plug-in-Gait model consisting of 39 markers was used, with the placement consistent with the Plug-in-

Gait model provided by VICON (see Figure 7.1a and Figure 2A.1 in Appendix 2). Participants wore only shorts or tight-fitting clothing after anthropometric measures described by the user manual were taken and entered into the software (Nexus 2.5, Bodybuilder, 'plug-in-gait model', VICON Motion Systems, Oxford). Fourteen VICON cameras (VICON MX T160, 2 Megapixel) were positioned on scaffolding which gave a ~170 m^3 viewing area and calibration was completed following the manufacturer's guidelines, such that the residual of measurement was < 0.01 mm. The Plug-in-Gait model predicts the position of joint centres from the available marker set and anthropometric measures with the equations described by Davis et al. (1991) used to calculate hip joint centres in both groups; the error of which is 6-10 mm in the anterior direction, 5-13 mm in the distal direction and 6-9 mm in the medial direction for controls (Harrington et al., 2007; Yousefi et al., 2014).

7.3.p1.3 Walking and running trials

Participants were asked to walk through the laboratory along a straight line (~10 m) to ensure movement was along the sagittal plane to the predefined 0,0,0 coordinates (Figure 7.1b). Two timing gates (1 m apart) were used to obtain walking speed. The same fixed walking speeds (0.56 - 1.94 $m \cdot s^{-1}$, step 0.28 $m \cdot s^{-1}$) and running (1.67 – 3.33 $m \cdot s^{-1}$, step 0.28 $m \cdot s^{-1}$) used in Chapter 4 were included (Chapter 4, Section 4.4.3). In addition, the same SSW speeds measured during $\dot{V}O_2$ assessment was also used. All participants were required to be within ±5% of each speed; trials that were outside these limits were discarded. Each participant completed each respective trial until at least three acceptable trials were recorded. For all trials, kinematic data were

recorded (100 Hz) when each participant were first and last detected as a whole-body by the software.

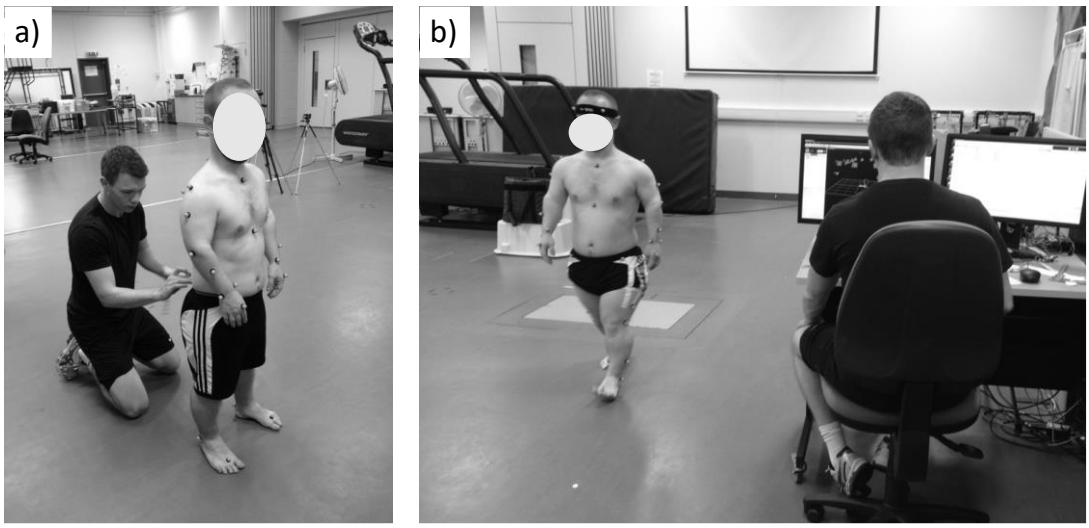

Figure 7.1: a) Plug-in-Gait marker set being positioned on a participant with Achondroplasia, and b) the laboratory set up (timing gates out of view) during a walking trial of a participant with Achondroplasia (grey panels on the floor denote the centre of the viewing area, i.e. coordinates 0, 0, 0).

7.3.p1.4 Spatial, temporal and kinematic calculations

Leg length (m) of all participants was measured as the distance from the anterior iliac spine to the medial malleolus of the ankle while standing using a measuring tape. Stride length (m) and stride frequency (Hz) were attained by observing the left and right heel marker in relation to the sagittal plane and floor. Based on Hof's NDN parameters of gait (Hof, 1996), stride length (stride length ÷ leg length) stride frequency (Equation 7.1) and speed (in the form of Froude's number (*Fr*), Equation 7.2) were calculated in both groups. Temporal events of heel contact and toe off for

the left and right sides were calculated as a percentage of total stride time. The double support phase was calculated as the overlap period of left and right foot floor contact for walking, while flight phase during running was determined as the time spent in the air during the stride. Seven discrete measures of the pelvis (P 1-7), six of the knee (K 1-6), and nine discrete measures of the hip (H 1-9) and ankle (A 1-9) were analysed in the sagittal, frontal and transverse planes (see Table 7.1) based on the recommendations of Benedetti et al. (1998). Further to the discrete measures, the average difference in joint kinematics between groups' pelvis, hip, knee and ankle position were determined over the entire stride for the sagittal plane only.

$$Equation\ 7.1: NDN\ frequency\ = \frac{Stride\ frequencey\ (Hz)}{\sqrt{9.81\ (m \cdot s^{-2})\ \div\ leg\ length\ (m)}}$$

$$Equation\ 7.2: Froude's\ Number\ = \frac{Gait\ velocity\ (m \cdot s^{-1})}{\sqrt{leg\ length\ (m)\ \cdot\ 9.81\ (m \cdot s^{-2})}}$$

7.3.p1.5 Centre of mass calculation

The segment masses measured by dual x-ray absorptiometry given in Chapter 2 were used alongside the inertial properties described by Dempster (1955) to predict CoM of both groups. In brief, the body was divided into 15 segments with the CoM calculated using the three-dimensional coordinates from the VICON system and given as an individual X and Y coordinate for each time frame during the gait cycle (Equation 7.3 and 7.4). The vertical position of the CoM was identified as an absolute measure from the floor. Vertical displacement of the CoM was calculated from its

position at initial heel contact to the positions at the local minima and maxima through the gait cycle. The medio-lateral displacement of the CoM was determined as the distance between its local minima and maxima in the transverse plane.

$$Equation\ 7.3: CoM_x = \frac{\sum_{i=1}^{N} m_i \cdot x_i}{m_B}$$

$$Equation\ 7.4: CoM_y = \frac{\sum_{i=1}^{N} m_i \cdot y_i}{m_B}$$

Where CoM_x and CoM_y are the respective horizontal (x) and vertical (y) coordinates of the CoM of the whole body, m_i is the mass of individual segments; x_i and y_i are the respective horizontal and vertical distances of the segments' centre of mass from the origin; and, m_B is the total-body mass of each participant.

Table 7.1: Descriptions of the discrete kinematic variables used to describe gait of the left leg only. Abbreviations given in the table are referred to in the text throughout.

Pelvis Kinematic Variables (°)		Knee Kinematic Variables (°)	
P1	Anterior pelvic tilt at initial heel contact	K1	Flexion at initial heel contact
P2	Peak anterior pelvic tilt during stance phase	K2	Peak flexion during stance phase
P3	Peak anterior pelvic tilt during swing phase	K3	Flexion at toe off
P4	Peak pelvic rise during stance phase	K4	Peak flexion during swing phase
P5	Peak pelvic drop during swing phase	K5	Varus angle at toe off
P6	Peak internal rotation during stride	K6	Peak varus angle during stance phase
P7	Peak external rotation during stride	K7	Peak varus angle during swing phase

Hip Kinematic Variables (°)		Ankle Kinematic Variables (°)	
H1	Flexion at initial heel contact	A1	Plantarflexion at initial heel contact
H2	Peak extension during stance phase	A2	Peak plantarflexion during stance phase
H3	Peak extension at toe off	A3	Plantarflexion at toe off
H4	Peak abduction during stance phase	A4	Peak eversion at heel contact
H5	Peak adduction during stance phase	A5	Peak abduction at heel contact
H6	Peak abduction during swing phase	A6	Peak eversion during stance phase
H7	Peak adduction during swing phase	A7	Peak abduction during stance phase
H8	Internal rotation at heel contact	A8	Eversion at toe off
H9	Internal rotation at toe off	A9	Abduction at toe off

7.3.p1.6 Gait profile score

Based on the method proposed by Baker et al. (2009) and presented in Baker et al. (2012), 15 gait specific RMS differences, known as Gait Variable Scores (GVSs, units °) were calculated for each group. Specifically, GVSs were calculated for: pelvic tilt, obliquity and rotation; hip flexion/extension, abduction/adduction and internal/external rotation; knee flexion/extension; ankle plantar/dorsiflexion; foot progression; and, a total GVS for each leg (Equation 7.5, (Baker et al., 2012)). For the GVSs of the group with Achondroplasia, RMS differences were compared to the control mean, whereas the control group's GVSs were compared to their own mean. GPS (units °) was then calculated as the sum of the RMS of each groups' 15 GVSs, given in Equation 7.6 (Baker et al., 2012). A worked example of knee flexion/extension GVS and GPS of one participant with Achondroplasia is given in Tables A3.1 and A3.2 in appendix 3, respectively.

$$Equation\ 7.5: GVS_i = \sqrt{\frac{1}{T}\sum_{t=1}^{T}(x_{i,t} - \bar{x}_{i,t}^{ref})^2}$$

$$Equation\ 7.6: GPS = \sqrt{\frac{1}{N}\sum_{i=1}^{N} GVS_i^2}$$

Where GVS_i is the i^{th} gait variable score for a specified joint, $x_{i,t}$ is each participant's gait variable (Achondroplasia and control), i, calculated at a specific time point, t,

and $\bar{x}_{i,t}^{ref}$ is the same variable averaged from the reference group (control only), T is the number of data points the gait cycle is divided into.

7.3.p1.7 Statistical analysis

Raw data exported from the VICON system were exported to Microsoft Excel (2000) and time normalised to 100 data points using a cubic spline interpolation method (Microsoft Excel macro, 2000). Statistical analyses were conducted using SPSS (IBM, v24). For absolute and NDN spatio-temporal parameters, discrete joint kinematic measures, CoM movement and the mean difference in joint angles throughout the gait cycle, data were assumed parametric following normality tests (Shaprio-Wilk) and equal variance tests (Levene's). To avoid the likelihood of Type I errors, a repeated measures ANOVA with a between group factor was used to identify between effects for all variables. Planned contrasts, in the form of independent t-tests were used to compare the above variables between groups, with data being presented as mean (SD).

For GPS related comparisons, a log-transformation of GVS was performed due to the skewed distribution of the data. To account for Type I errors, Mann Whitney-U tests were conducted to identify effects between groups' individual GVSs and GPS. Wilcoxon tests were performed within each group to identify effects between left and right legs for each GVS, with data presented as median (interquartile range, IQR). Each GVS was inferentially compared between groups and presented graphically as

a collection of RMS differences known as the Movement Analysis Profile (MAP) (Baker et al., 2009).

Lastly, the speed of each walking and running trial across the floor was compared to the treadmill-based speeds from Chapter 4 using a repeated measures ANOVA. Alpha was set to ≤ 0.05 for all tests.

Part 2: Walking results

7.4.p2.1 Spatial-temporal

7.4.p2.1.1 Gait speed

There was no difference in either groups' walking or running speed across the floor compared to those measured during $\dot{V}O_2$ assessment on a motorised treadmill (Chapter 4, $P > 0.05$). The group with Achondroplasia were 23% slower at SSW compared to controls (Achondroplasia, 1.02 (0.13) $m \cdot s^{-1}$; control 1.33 (0.14) $m \cdot s^{-1}$, $P < 0.001$). When presented as NDN *Fr* values, the group with Achondroplasia were quicker at every walking speed than controls ($P < 0.001$) other than SSW where there was no difference between groups ($P = 0.466$, Table 7.2).

7.4.p2.1.2 Stride length

The group with Achondroplasia had, on average, a 25% shorter stride length across all speeds compared to controls ($P < 0.001$, Figure 7.2a). At SSW, the group with Achondroplasia had a 30% shorter stride length than controls ($P < 0.001$, Figure 7.2a). When stride length was presented as NDN values (normalising for leg length), the group with Achondroplasia had a longer stride than controls at every walking speed ($P < 0.05$, Table 7.2).

7.4.p2.1.3 Stride frequency

The group with Achondroplasia had, on average, a 23% higher stride frequency across all speeds compared to controls ($P < 0.001$, Figure 7.2b), indicating a 13% greater stride frequency at SSW compared to controls ($P < 0.001$, Figure 7.2b). When

presented as NDN values, there was no difference in stride frequency between groups ($P > 0.05$), other than SSW where the group with Achondroplasia had a lower stride frequency than controls ($P < 0.001$, Table 7.2).

7.4.p2.1.4 Temporal measures

The group with Achondroplasia had a shorter stride time than controls at every walking speed ($P < 0.001$, Figure 7.2c). When temporal events were normalised to stance time however, there were no between group differences in time to left toe off ($P = 0.612$) or right heel contact at any speed ($P = 0.418$, Table 7.2).

7.4.p2.2 Discrete kinematic variables

7.4.p2.2.1 Pelvis

Significant effects were observed in all pelvis measures (P1-3 and P6-7, $P < 0.05$) other than P4 and P5 ($P > 0.05$, Table A4.1 in Appendix 4). The group with Achondroplasia exhibited a greater anterior pelvic tilt at heel contact (P1) within the stance phase (P2) and a greater peak anterior pelvic tilt within the swing phase (P3) compared to controls at all walking speeds ($P < 0.001$, Figure 7.3 and Table A4.1 in Appendix 4). A greater peak internal rotation of the pelvis during the stride (P6) at walking speeds 1.11 to 1.96 $m \cdot s^{-1}$ was observed in the group with Achondroplasia compared to controls ($P < 0.001$, Figure 7.3 and Table A4.1 in Appendix 4). The group with Achondroplasia also had a greater peak external rotation of the pelvis during the stride (P7) compared to controls at walking speeds 1.11 to 1.96 $m \cdot s^{-1}$ ($P < 0.001$,

Figure 7.3 and Table A4.1 in Appendix 4). A significant effect was observed in the mean difference of the pelvis ($P < 0.001$) with the group with Achondroplasia being more anteriorly tilted throughout the stride at every walking speed compared to controls ($P < 0.01$, Table 7.3 and Table A4.1 in Appendix 4).

7.4.p2.2.2 Hip

There were no differences in any hip measure (H1-7 and H9, $P > 0.05$) other than the group with Achondroplasia being more internally rotated at heel contact than controls at every speed ($P < 0.001$, Figure 7.4 and Table A4.2 in Appendix 4). A significant effect was observed in the average mean difference of the hip ($P = 0.014$) with the group with Achondroplasia being more flexed throughout the stride at every walking speed compared to controls ($P \leq 0.05$, Table 7.4 and Table A4.2 in Appendix 4).

7.4.p2.2.3 Knee

A significant effect was found for K2-4 and K6-7 only ($P < 0.05$, Table A4.3 in Appendix 4). The group with Achondroplasia had greater peak knee flexion during stance (K2) and greater knee flexion at toe off (K3) than controls for all walking speeds other than 1.67 $m \cdot s^{-1}$ (Figure 7.5 and Table A4.3 in Appendix 4). A greater peak knee flexion during swing (K4) was observed in the group with Achondroplasia compared to controls at all walking speeds (Figure 7.5 and Table A4.3 in Appendix 4). The group with Achondroplasia also had a greater peak varus knee position during stance at speeds 1.39 – 1.96 $m \cdot s^{-1}$ and SSW only, but had a lower peak varus position of the

knee during swing for all walking speeds compared to controls (Figure 7.5 and Table A4.3 in Appendix 4). A significant effect was also observed in the average mean difference of the knee ($P < 0.001$) with the group with Achondroplasia being more flexed throughout the stride at every walking speed compared to controls ($P < 0.01$, Table 7.3 and Table A4.3 in Appendix 4).

7.4.p2.2.4 Ankle

The group with Achondroplasia had less plantarflexion at heel contact (A1) and at toe off (A2) at all speeds, and less peak eversion during stance (A6) at speeds 1.39 – 1.96 $m \cdot s^{-1}$ only compared to controls (Figure 7.6 Table A4.4 in Appendix 4). A significant effect was observed in the average mean difference of the ankle ($P < 0.001$) with the group with Achondroplasia being more dorsiflexed throughout the stride at every walking speed compared to controls ($P < 0.001$, Table 7.3 and Table A4.4 in Appendix 4).

7.4.p2.3 Centre of mass movement

7.4.p2.3.1 Vertical movements

As an absolute measure, the CoM height of the group with Achondroplasia was lower at heel contact, local maxima during left stance, local minima during double support and, local maxima during right stance than controls at all speeds ($P < 0.001$, Table 7.4). The vertical displacement of the CoM from initial heel contact was less in the group with Achondroplasia than controls' during left stance phase at all walking

speeds ($P < 0.05$) other than 0.56 $m \cdot s^{-1}$ ($P = 0.262$, Table 7.5 and Table A4.5 in Appendix 4). The vertical displacement of the CoM from initial heel contact was also less in the group with Achondroplasia compared to controls' during right stance phase at all walking speeds ($P < 0.05$) other than 0.56 and 0.83 $m \cdot s^{-1}$ ($P = 0.491$ and $P = 0.561$ respectively, Table 7.5 and Table A4.5 in Appendix 4). There was no difference in vertical displacement from initial heel contact during double support between groups ($P = 0.347$).

7.4.p2.3.2 Medio-lateral movements

The group with Achondroplasia had a greater medio-lateral displacement of the CoM at speeds 1.67, 1.94 $m \cdot s^{-1}$ and SSW only ($P < 0.05$, Table 7.5 and Table A4.5 in Appendix 4).

7.4.p2.4 Gait profile score

There was no difference between the left and right leg GVSs within each group ($P > 0.05$), but the group with Achondroplasia had a higher GVS at all joints ($P < 0.05$) other than foot internal/external rotation ($P > 0.05$, Figure 7.7). The group with Achondroplasia had a higher anterior/posterior tilt GVS than controls at speeds 0.56 ($P = 0.048$), 1.39 ($P = 0.008$), 1.67 ($P = 0.003$), 1.94 $m \cdot s^{-1}$ ($P = 0.002$) and SSW ($P = 0.051$) but no difference was found at speeds 0.83 ($P = 0.057$) and 1.11 $m \cdot s^{-1}$ compared to controls ($P = 0.079$, Figure 7.7). A higher hip flexion/extension, knee flexion/extension and ankle planta/dorsiflexion GVS was observed in the group with Achondroplasia at every speed compared to controls ($P < 0.001$, Figure 7.7). The

group with Achondroplasia also had a higher pelvic obliquity GVS at speeds 0.56 ($P = 0.026$), 1.11 ($P = 0.020$), 1.39 ($P < 0.001$), 1.67 ($P = 0.027$), 1.94 $m \cdot s^{-1}$ ($P < 0.001$) and SSW ($P = 0.001$) compared to controls, but no difference was found at 0.83 $m \cdot s^{-1}$ ($P = 0.074$). Hip adduction/abduction and internal/external rotation GVSs were higher in the group with Achondroplasia at every walking speed compared to controls ($P < 0.001$, Figure 7.6). The total GVS for the respective left and right leg was higher in the group with Achondroplasia at each speed compared to controls ($P < 0.001$, Figure 7.7). The combination of the GVSs led to the GPS in the group with Achondroplasia being higher than controls at all speeds ($P \leq 0.001$, Figure 7.7).

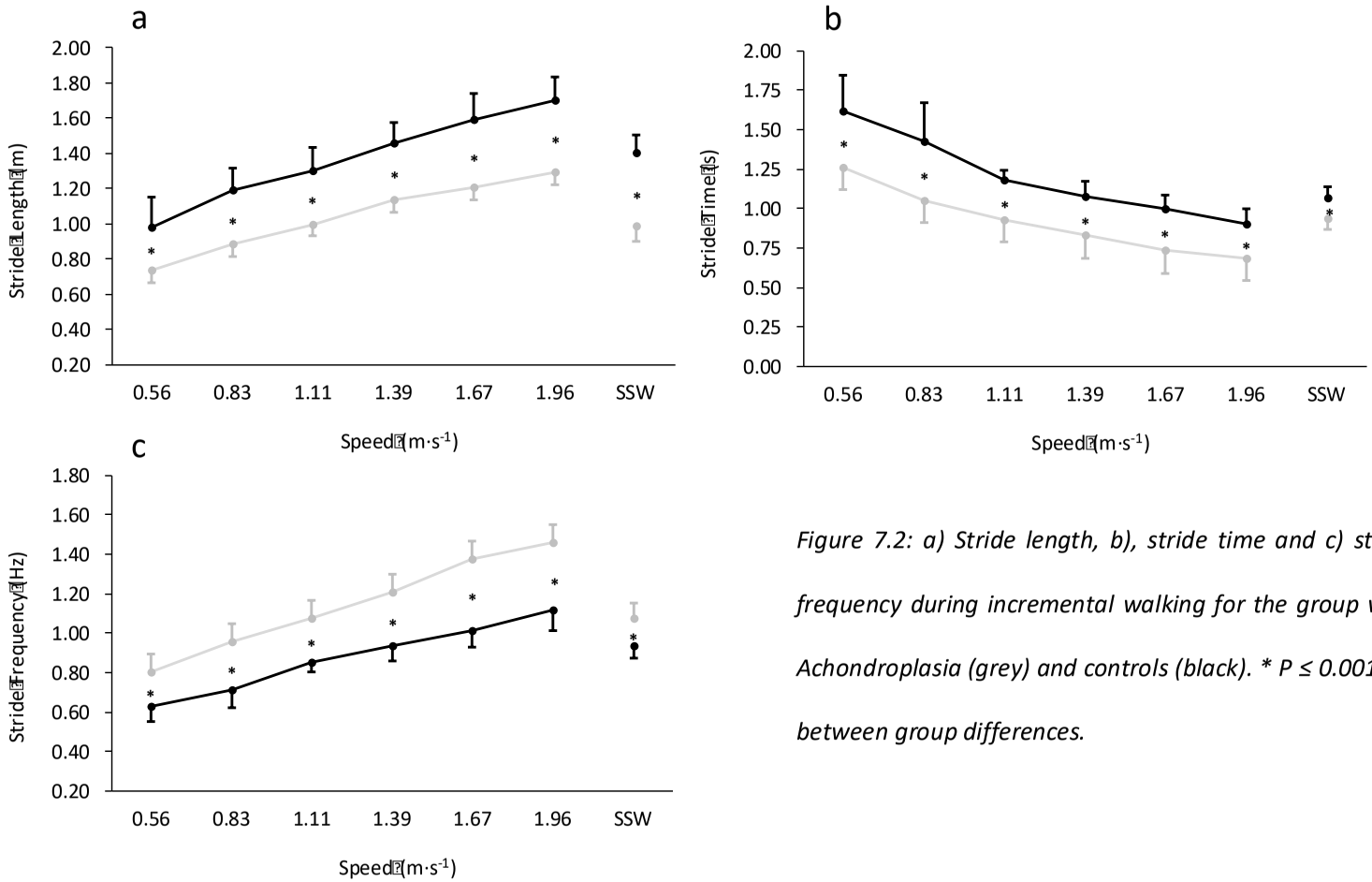

*Figure 7.2: a) Stride length, b), stride time and c) stride frequency during incremental walking for the group with Achondroplasia (grey) and controls (black). * P ≤ 0.001 for between group differences.*

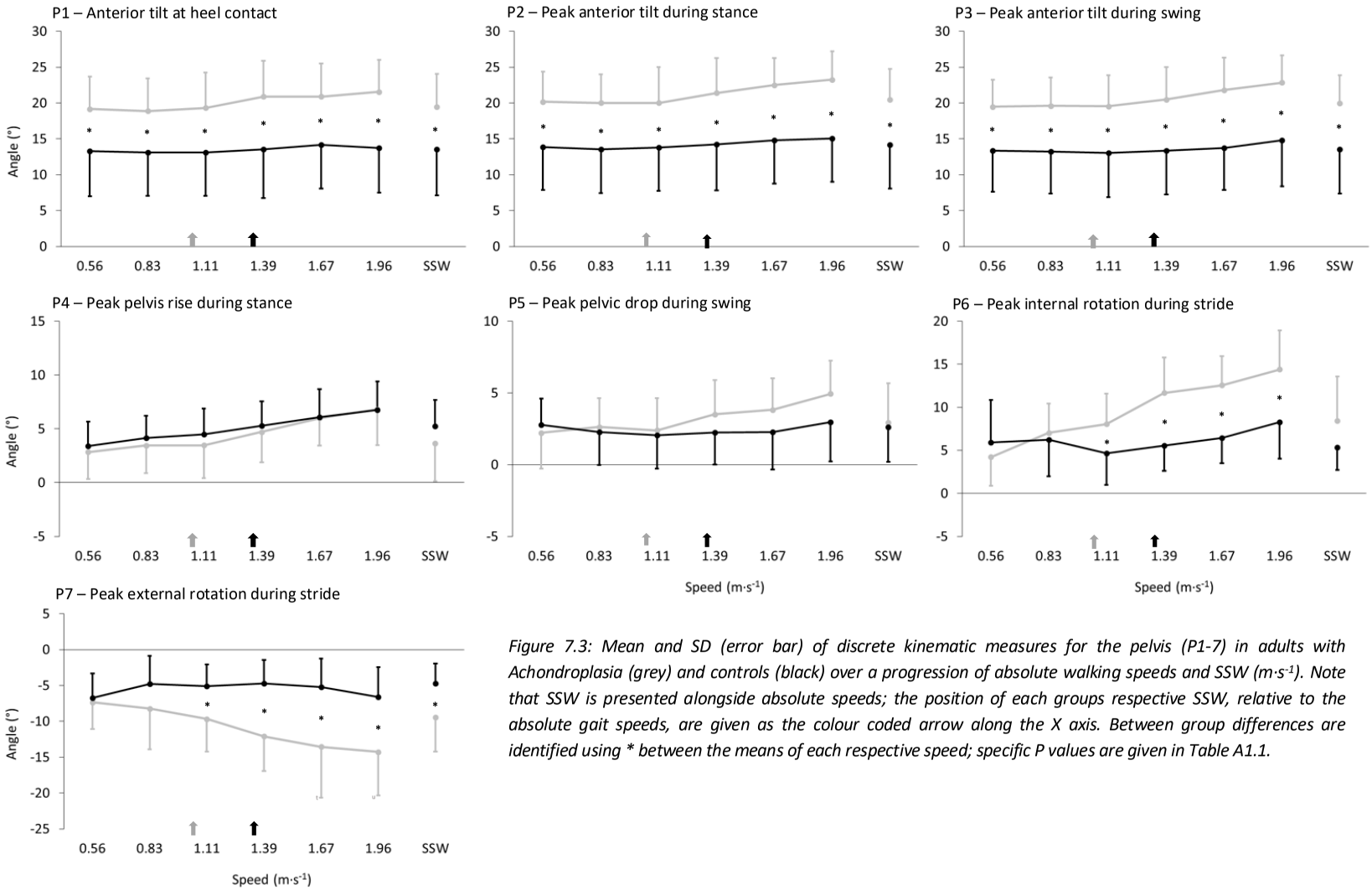

*Figure 7.3: Mean and SD (error bar) of discrete kinematic measures for the pelvis (P1-7) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute walking speeds and SSW (m·s^{-1}). Note that SSW is presented alongside absolute speeds; the position of each groups respective SSW, relative to the absolute gait speeds, are given as the colour coded arrow along the X axis. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A1.1.*

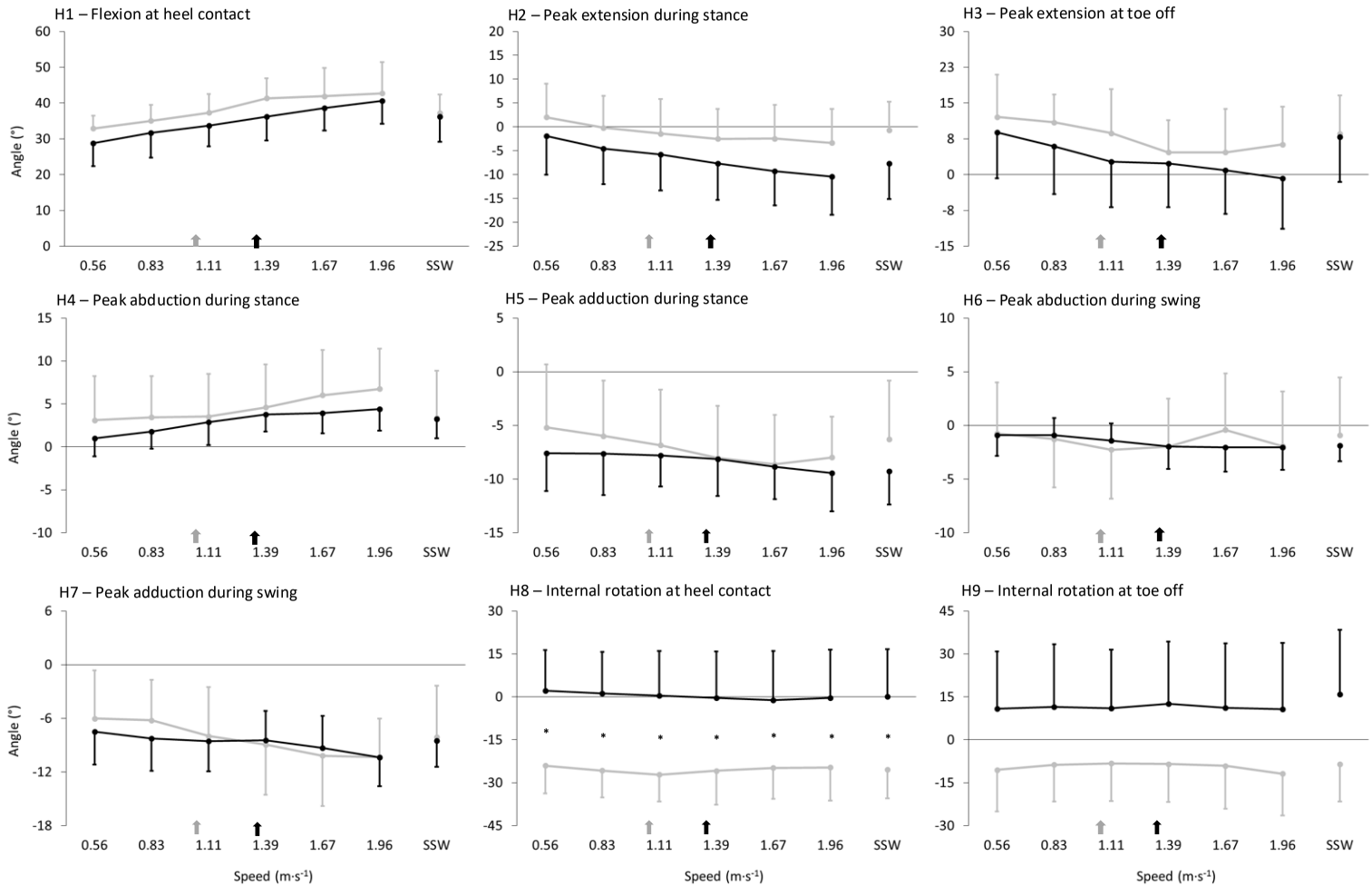

*Figure 7.4: Mean and SD (error bar) of discrete kinematic measures for the hip (H1-9) over a progression of absolute walking speeds and SSW ($m \cdot s^{-1}$) for adults with Achondroplasia (grey) and controls (black) Note that SSW is presented alongside absolute speeds; the position of each groups respective SSW, relative to the absolute gait speeds, are given as the colour coded arrow along the X axis. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A1.2.*

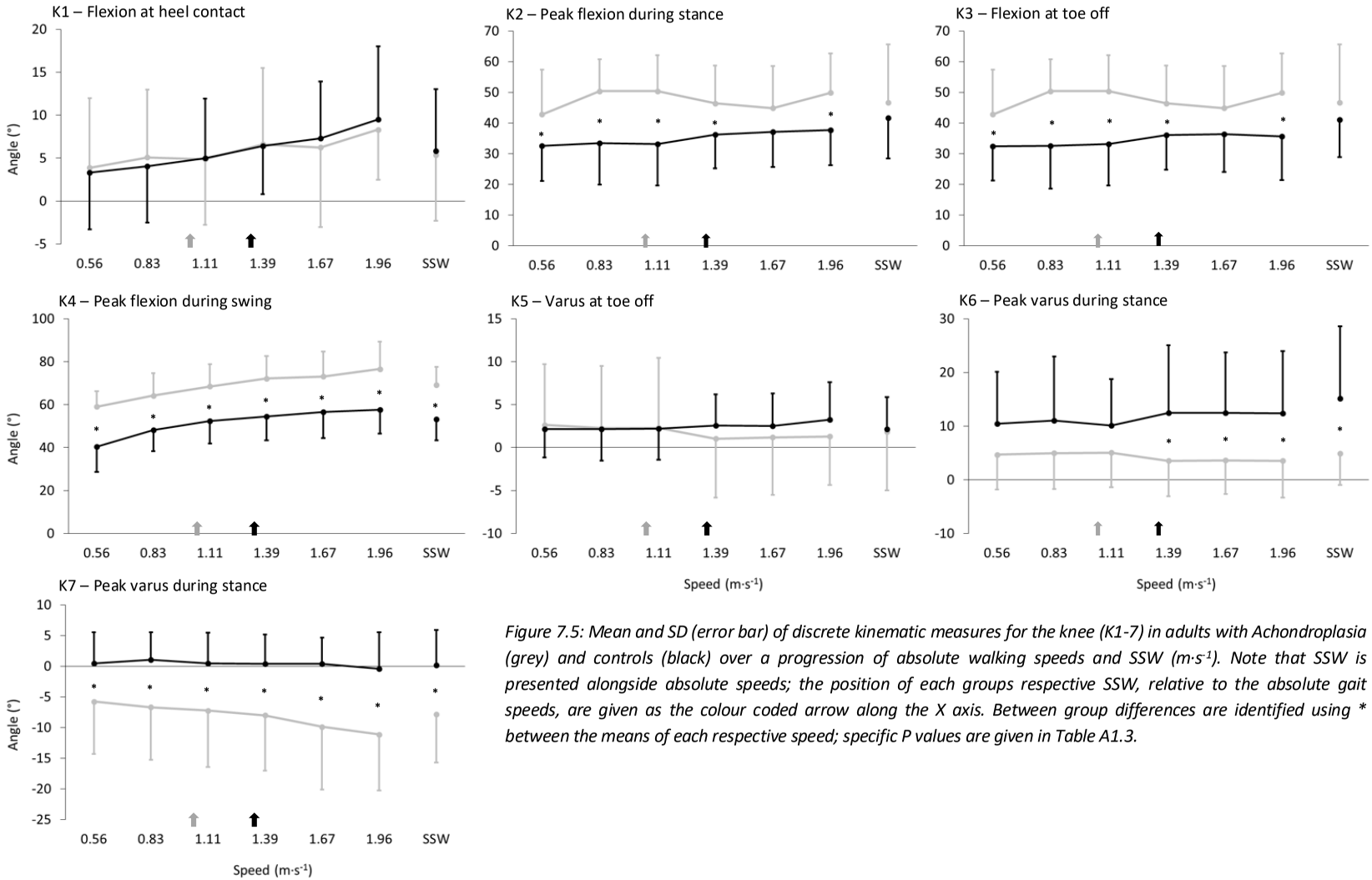

*Figure 7.5: Mean and SD (error bar) of discrete kinematic measures for the knee (K1-7) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute walking speeds and SSW (m·s^{-1}). Note that SSW is presented alongside absolute speeds; the position of each groups respective SSW, relative to the absolute gait speeds, are given as the colour coded arrow along the X axis. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A1.3.*

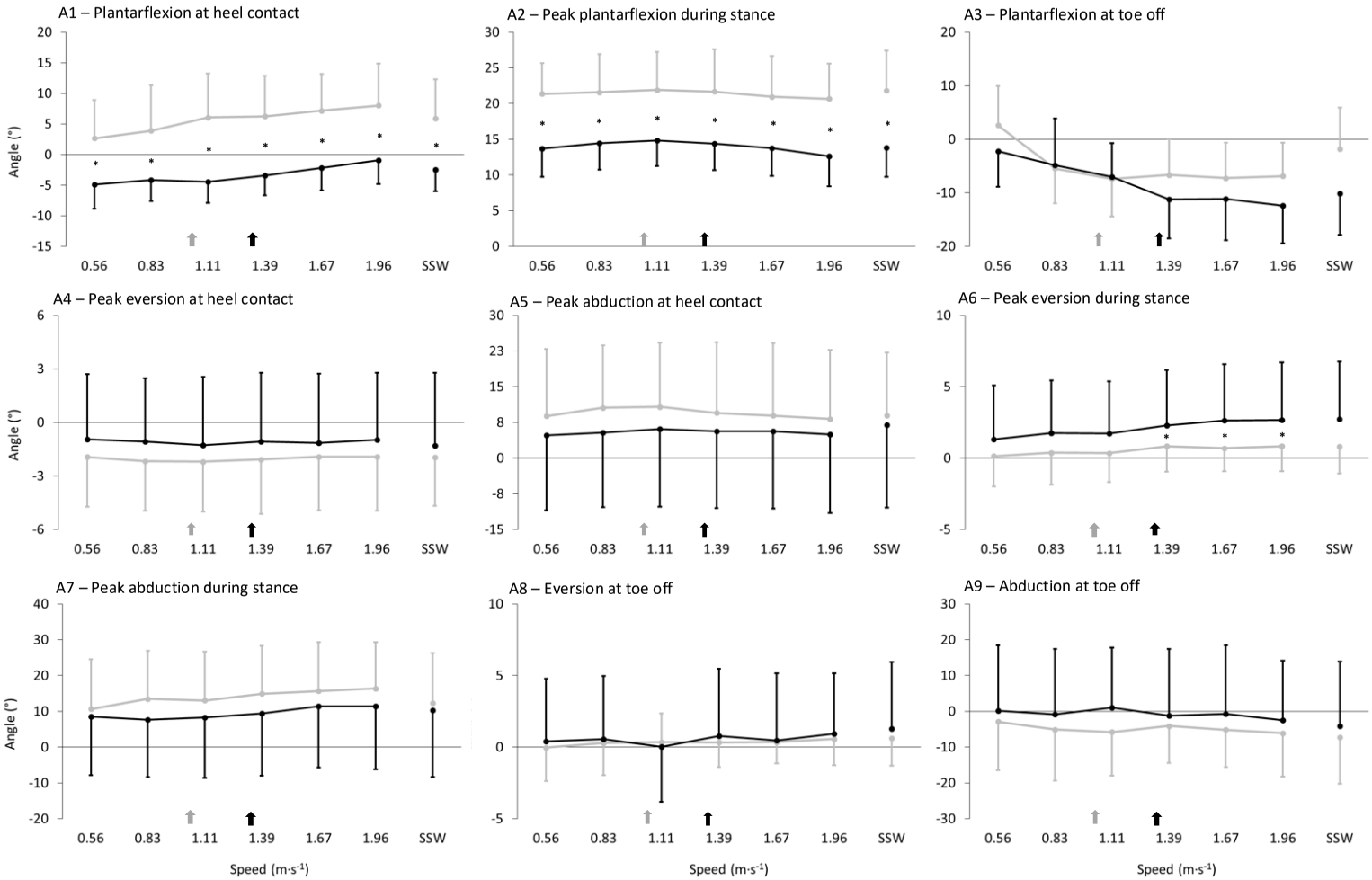

*Figure 7.6: Mean and SD (error bar) of discrete kinematic measures for the ankle (A1-9) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute walking speeds and SSW (m·s⁻¹). Note that SSW is presented alongside absolute speeds; the position of each groups respective SSW, relative to the absolute gait speeds, are given as the colour coded arrow along the X axis. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A1.4.*

Table 7.2: Mean (SD) absolute (% of stride time) and dimensionless temporal gait measures for the group with Achondroplasia and controls at incremental walking speeds ($m \cdot s^{-1}$).

	0.56		0.83		1.11		1.39		1.67		1.96		SSW	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
LTO	67 (3)	66 (19)	64 (2)	61 (16)	62 (1)	63 (5)	59 (2)	61 (4)	54 (10)	60 (5)	58 (3)	58 (5)	63 (4)	64 (5)
RHC	48 (7)	48 (3)	48 (6)	48 (3)	46 (6)	50 (4)	46 (5)	48 (3)	49 (4)	48 (3)	48 (4)	49 (4)	46 (9)	48 (3)
$\boldsymbol{\lambda}$	1.25 (0.13) †	1.03 (0.17)	1.50 (0.17) ‡	1.25 (0.12)	1.69 (0.13) ‡	1.37 (0.14)	1.92 (0.12) ‡	1.54 (0.14)	0.49 (0.03) ‡	2.04 (0.14)	2.19 (0.08) ‡	1.80 (0.14)	1.68 (0.16) ‡	1.48 (0.09)
f	0.20 (0.02)	0.20 (0.03)	0.23 (0.02)	0.22 (0.03)	0.26 (0.02)	0.26 (0.02)	0.30 (0.01)	0.29 (0.02)	0.34 (0.03)	0.31 (0.02)	0.36 (0.02)	0.35 (0.03)	0.26 (0.02) ‡	0.29 (0.02)
Fr	0.06 (0.01) †	0.04 (0.02)	0.12 (0.01) ‡	0.08 (0.02)	0.20 (0.03) ‡	0.13 (0.03)	0.32 (0.03) ‡	0.20 (0.03)	0.47 (0.04) ‡	0.28 (0.05)	0.62 (0.07) ‡	0.39 (0.07)	0.20 (0.05)	0.19 (0.03)

A, Achondroplasia; C, control; LTO, Left Toe Off; RHC, Right Heel Contact; $\boldsymbol{\lambda}$, dimensionless stride length; f, dimensionless stride frequency; Fr, Froude's number; † $P < 0.01$, ‡ $P < 0.001$

Table 7.3: Average joint positions (°) of the pelvis, hip, knee and ankle in the sagittal plane only for group with Achondroplasia and controls during incremental walking speeds ($m \cdot s^{-1}$). Values given as mean (SD).

	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
Pelvis	$P < 0.001$	6.6 (3.0) ‡	6.2 (2.5) ‡	6.0 (3.1) ‡	6.3 (3.6) ‡	6.3 (3.2) ‡	6.3 (2.8) ‡	6.5 (2.2) ‡
Hip	$P = 0.014$	4.7 (1.3) †	6.0 (1.6) †	4.3 (1.3) *	5.5 (1.5) *	6.1 (1.8) *	5.2 (3.3) *	4.4 (2.0) *
Knee	$P < 0.001$	8.9 (5.5) ‡	8.6 (4.9) ‡	9.1 (4.8) ‡	10.7 (5.8) ‡	10 (5.7) ‡	10 (7.7) ‡	8.1 (5.3) †
Ankle	$P < 0.001$	6.0 (0.3) ‡	7.2 (0.5) ‡	5.7 (0.6) ‡	6.5 (0.5) ‡	6.8 (0.8) ‡	7.1 (1.0) ‡	5.9 (0.5) ‡

ME, Main effect; * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$

Table 7.4: Absolute vertical displacement of the body's centre of mass from initial heel contact during incremental walking speeds ($m \cdot s^{-1}$) in the group with Achondroplasia and controls. Values shown as mean (SD).

	0.56		0.83		1.11		1.39		1.67		1.96		SSW	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
Height at Initial Heel Contact (m)	0.72 (0.02)	1.01 (0.05)	0.72 (0.02)	1.00 (0.04)	0.71 (0.02)	1.00 (0.05)	0.71 (0.02)	0.99 (0.05)	0.71 (0.02)	0.99 (0.04)	0.71 (0.02)	0.99 (0.04)	0.71 (0.02)	0.99 (0.04)
Max Height During Left Stance (m)	0.73 (0.02)	1.03 (0.05)	0.73 (0.02)	1.03 (0.05)	0.73 (0.02)	1.03 (0.05)	0.73 (0.02)	1.03 (0.05)	0.73 (0.02)	1.03 (0.04)	0.73 (0.02)	1.03 (0.04)	0.73 (0.02)	1.02 (0.04)
Min Height During Double Support (m)	0.72 (0.02)	1.01 (0.05)	0.72 (0.02)	1.00 (0.04)	0.71 (0.02)	1.00 (0.05)	0.71 (0.02)	0.99 (0.05)	0.71 (0.02)	0.99 (0.05)	0.71 (0.02)	0.98 (0.05)	0.71 (0.02)	0.99 (0.04)
Max Height During Right Stance (m)	0.74 (0.02)	1.03 (0.05)	0.74 (0.02)	1.03 (0.05)	0.74 (0.02)	1.03 (0.05)	0.73 (0.02)	1.03 (0.05)	0.73 (0.02)	1.03 (0.04)	0.73 (0.02)	1.03 (0.04)	0.74 (0.02)	1.02 (0.04)

A, Achondroplasia; C, Control; Differences between group for same speed only; * $P \leq 0.001$.

Table 7.5: Medio-lateral and vertical displacement of the body's centre of mass from initial heel contact during incremental walking speeds ($m \cdot s^{-1}$) in the group with Achondroplasia and controls. Values shown as mean (SD).

	0.56		0.83		1.11		1.39		1.67		1.96		SSW	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
Total left-to-right displacement (m)	0.09 (0.02)	† 0.08 (0.03)	0.09 (0.03)	† 0.08 (0.03)	0.08 (0.02)	* 0.06 (0.03)	0.08 (0.02)	* 0.05 (0.02)	0.07 (0.02)	* 0.06 (0.02)	0.08 (0.02)	* 0.06 (0.03)	0.09 (0.02)	* 0.07 (0.02)
Max Height During Left Stance (m)	0.02 (0)	0.02 (0.01)	0.02 (0.01)	‡ 0.02 (0)	0.02 (0.01)	* 0.03 (0.01)	0.03 (0.01)	‡ 0.03 (0.01)	0.02 (0.02)	† 0.04 (0.01)	0.02 (0.01)	* 0.04 (0.01)	0.02 (0.01)	* 0.03 (0.01)
Min Height During Double Support (m)	0.00 (0)	0.00 (0)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	-0.01 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0.01)	0.00 (0)
Max Height During Right Stance (m)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	‡ 0.03 (0.01)	0.03 (0.01)	‡ 0.03 (0.01)	0.02 (0.02)	† 0.04 (0.01)	0.02 (0.02)	* 0.04 (0.01)	0.02 (0.01)	† 0.03 (0.01)

A, Achondroplasia; C, Control; Differences between group for same speed only; ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.

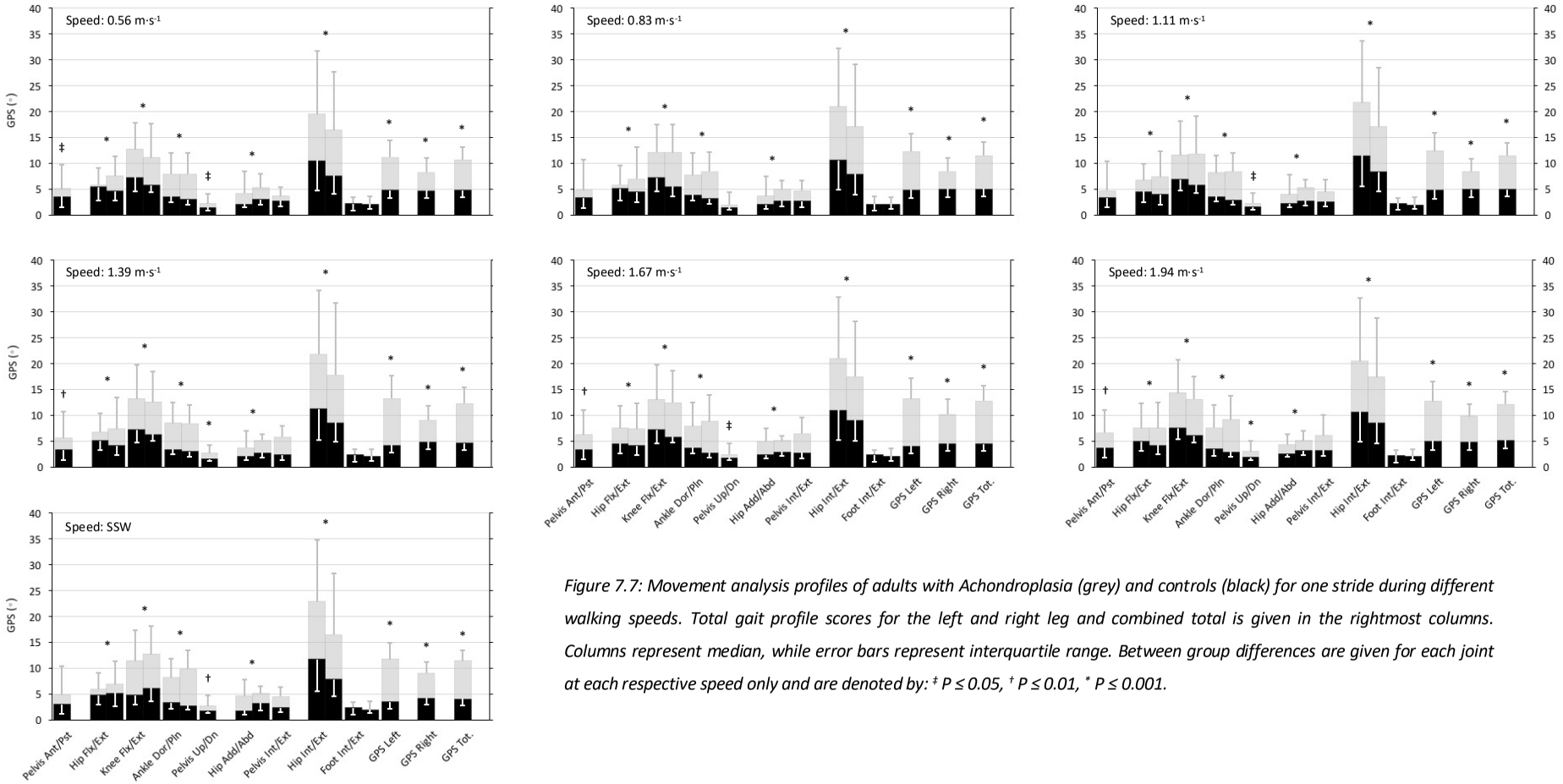

*Figure 7.7: Movement analysis profiles of adults with Achondroplasia (grey) and controls (black) for one stride during different walking speeds. Total gait profile scores for the left and right leg and combined total is given in the rightmost columns. Columns represent median, while error bars represent interquartile range. Between group differences are given for each joint at each respective speed only and are denoted by: ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.*

Part 3: Running results

7.5.p3.1 Spatial-temporal

7.5.p3.1.1 Gait speed

There were no differences between groups' speeds measured using timing gates, compared to those measured during $\dot{V}O_2$ assessment on a motorised treadmill ($P > 0.05$). When presented as NDN *Fr* values, the group with Achondroplasia were quicker at every running speed than controls ($P < 0.001$, Table 7.6).

7.5.p3.1.2 Stride length

The group with Achondroplasia had, on average, a 24% shorter stride length at every running speed compared to controls ($P < 0.001$, Figure 7.8a). When presented as NDN values, the group with Achondroplasia had a longer stride at every running speed compared to controls ($P < 0.001$, Table 7.6).

7.5.p3.1.3 Stride frequency

The group with Achondroplasia had, on average, a 21% higher stride frequency at every absolute speed compared to controls ($P < 0.001$, Figure 7.8b). There was no difference in NDN stride frequency between groups at any running speed ($P > 0.05$, Table 7.6).

7.5.p3.1.4 Temporal measures

The group with Achondroplasia had a 21% average shorter stride time than controls at every walking speed, ($P < 0.001$, Figure 7.8c). When temporal events were normalised to stance time however, there were no differences in time to left toe off, right heel contact or right toe off for any running speed ($P > 0.05$, Table 7.6).

7.5.p3.2 Discrete kinematic variables

7.5.p3.2.1 Pelvis

A between group effect was observed at P7 only ($P = 0.013$, Table A2.7 in Appendix 2). The group with Achondroplasia had a greater peak external rotation of the pelvis during the stride (P7) compared to controls at running speeds 1.94, 2.22, 2.78 and 3.33 $m \cdot s^{-1}$ only ($P < 0.05$, Figure 7.9 and Table A4.6 in Appendix 4). There was a significant group effect of the average position of the pelvis through the stride ($P = 0.038$) with the group with Achondroplasia being more anteriorly tilted throughout the stride at every running speed compared to controls ($P < 0.01$, Table 7.7 and Table A4.6 in Appendix 4).

7.5.p3.2.2 Hip

There was a significant between group effect at the hip for measures H8 and H9 only ($P < 0.001$ and $P = 0.008$ respectively, Table A4.7 in Appendix 4). Greater internal rotation of the hip at heel contact (H8, $P < 0.001$) and at toe off (H9, $P < 0.001$) was observed in the group with Achondroplasia compared to controls at every running

speed (Figure 7.10 and Table A4.7 in Appendix 4). The average mean difference in hip angle was the same between groups when running (P = 0.776, Table 7.7 and Table A4.7 in Appendix 4).

7.5.p3.2.3 Knee

A between group main effect was found for K3 and K5-7 only (P < 0.05, Table A4.8 in Appendix 4). The group with Achondroplasia had more knee flexion at toe off (K3) at running speeds 2.22 and 2.78 to 3.33 $m \cdot s^{-1}$ only (Figure 7.11 and Table A4.8 in Appendix 4). A greater peak varus knee position at toe off (K5) for all running speeds (P < 0.05) and a larger peak varus knee position during the stance phase (K6) at running speeds 1.94 – 3.33 $m \cdot s^{-1}$ (P < 0.05) was observed in the group with Achondroplasia compared to controls. However, a lower peak varus position of the knee during swing was observed in the group with Achondroplasia for all running speeds, compared to controls (P < 0.05, Figure 7.11 and Table A4.8 in Appendix 4). The average mean difference in knee angle was the same between groups when running (P = 0.082, Table 7.7 and Table A4.8 in Appendix 4).

7.5.p3.2.4 Ankle

There was a between group effect for A5 and A6 only (P < 0.001 and P = 0.008 respectively). The group with Achondroplasia had a greater peak abduction of the ankle at heel contact (A5) and a lower peak eversion of the ankle during stance (A6) compared to controls at every running speed (P < 0.05, Figure 7.12 and Table A4.9 in Appendix 4). A significant effect was observed in the average mean difference of the

ankle (P = 0.014) with the group with Achondroplasia being more dorsiflexed throughout the stride at every running speed compared to controls (P < 0.05, Table 7.7 and Table A4.9 in Appendix 4).

7.5.p3.3 Centre of mass movement

7.5.p3.3.1 Vertical movements

A significant between group effect existed for absolute CoM height at all running speeds (P < 0.001) with the position of the CoM being lower in the group with Achondroplasia than controls at heel contact, local minima during left stance, local maxima during first flight, local minima during right stance and local maxima during second flight, at all speeds (P < 0.001, Table 7.8). The CoM displaced less from heel contact to local minima during left stance at all running speeds in the group with Achondroplasia compared to controls (P < 0.01, Table 7.8 and Table A4.10 in Appendix 4), but only displaced less during the right stance at running speeds 1.96, 2.22, 2.56 and 3.06 $m \cdot s^{-1}$ compared to controls (P < 0.05, Table 7.8 and Table A4.10 in Appendix 4). The CoM of the group with Achondroplasia displaced less from heel contact to local maxima of the first flight phase at running speeds 1.67, 1.96, 2.78 and 3.33 $m \cdot s^{-1}$ only (P < 0.05, Table 7.8 and Table A4.10 in Appendix 4), but displaced less from heel contact to local maxima of the right stance phase at every speed compared to controls (P ≤ 0.01, Table 7.8 and Table A4.10 in Appendix 4).

7.5.p3.3.2 Medio-lateral movements

There was no difference in the total left-to-right displacement of each group's CoM at any speed (P = 0.551, Table 7.8 and Table A4.10 in Appendix 4).

7.5.p3.4 Gait profile score

There was no difference between the left and right leg GVSs within each group (P > 0.05), but the group with Achondroplasia had a higher GVS at all joints (P < 0.05) other than planta/dorsiflexion (P = 0.092) and foot internal/external rotation (P = 0.056, Figure 7.13). The group with Achondroplasia had more anterior/posterior tilt GVS during running at 3.06 $m \cdot s^{-1}$ only, compared to controls (P = 0.017, Figure 7.13). The group with Achondroplasia had more hip flexion/extension GVS at all running speeds compared to controls (P < 0.05) other than 2.22 and 2.50 $m \cdot s^{-1}$ (P = 0.360 and P = 0.076, respectively, Figure 7.13). The group with Achondroplasia had more knee flexion/extension GVS than controls at running speeds 1.67 and 1.94 $m \cdot s^{-1}$ only (P = 0.005 and P = 0.022, respectively, Figure 7.13). More pelvis up/down, hip abduction/adduction and hip internal/external rotation GVS was observed in the group with Achondroplasia than controls at all running speeds (P < 0.05, Figure 7.13). The group with Achondroplasia had more pelvis internal/external rotation GVS than controls at all running speeds (P < 0.05) other than 2.50 $m \cdot s^{-1}$ (P = 0.56, Figure 7.13). The group with Achondroplasia had a greater GVS total in for the left and right leg than controls at all speeds (P < 0.05) other than 2.50 to 3.06 $m \cdot s^{-1}$ for the left leg (P > 0.05) and at 2.50 and 2.78 $m \cdot s^{-1}$ for the right leg (P > 0.05, Figure 7.13). GPS was higher in the group with Achondroplasia at ever running speed compared to controls

($P < 0.05$) other than 2.50 and 2.78m·s^{-1} ($P = 0.546$ and $P = 0.237$, respectively, Figure 7.13).

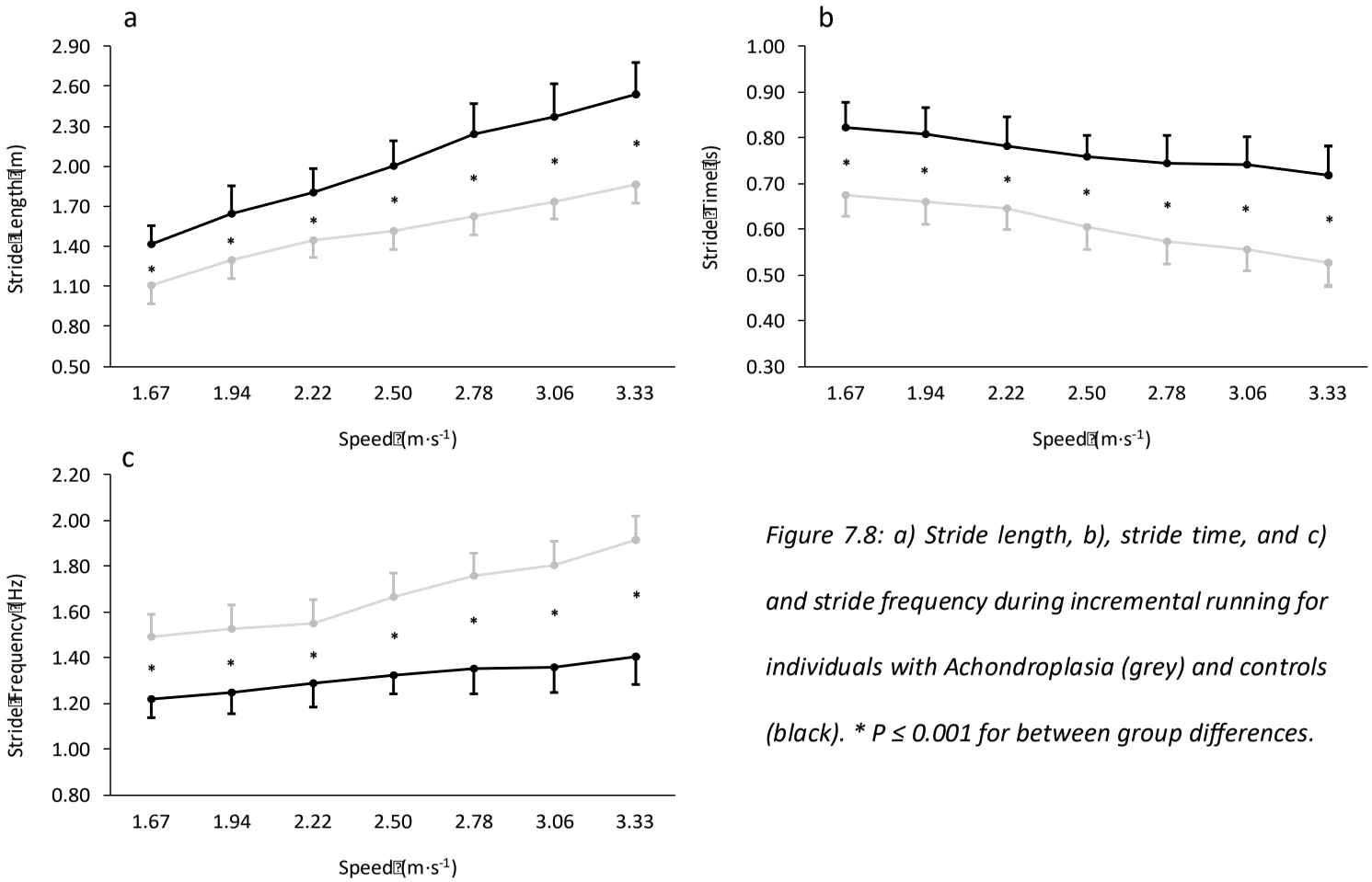

*Figure 7.8: a) Stride length, b), stride time, and c) and stride frequency during incremental running for individuals with Achondroplasia (grey) and controls (black). * P ≤ 0.001 for between group differences.*

Table 7.6: Mean (SD) absolute (% of stride time) and dimensionless temporal gait measures at incremental rnning speeds ($m \cdot s^{-1}$) in the group with Achondroplasia and controls.

	1.67		1.94		2.22		2.50		2.78		3.06		3.33	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
LTO	48 (6)	48 (10)	45 (6)	41 (7)	36 (14)	39 (6)	39 (3)	38 (7)	37 (4)	35 (5)	34 (4)	35 (4)	34 (3)	33 (6)
RHC	46 (3)	48 (4)	49 (4)	47 (4)	46 (3)	48 (4)	46 (4)	48 (3)	47 (4)	46 (3)	47 (3)	46 (4)	46 (4)	45 (5)
RTO	94 (5)	86 (23)	92 (8)	87 (6)	88 (6)	84 (11)	88 (4)	85 (5)	85 (3)	82 (5)	83 (4)	80 (6)	80 (5)	79 (7)
$\boldsymbol{\lambda}$	1.88 (0.28) ‡	1.50 (0.15)	2.20 (0.30) ‡	1.74 (0.24)	2.46 (0.32) ‡	1.91 (0.22)	2.57 (0.31) ‡	2.12 (0.23)	2.76 (0.23) ‡	2.36 (0.26)	2.95 (0.21) ‡	2.51 (0.29)	3.16 (0.33) †	2.68 (0.29)
f	0.37 (0.03)	0.38 (0.03)	0.37 (0.03)	0.39 (0.03)	0.38 (0.02)	0.40 (0.03)	0.41 (0.03)	0.41 (0.03)	0.43 (0.03)	0.39 (0.11)	0.44 (0.03)	0.42 (0.03)	0.47 (0.05) †	0.44 (0.04)
Fr	0.47 (0.12) ‡	0.32 (0.04)	0.68 (0.16) ‡	0.45 (0.13)	0.88 (0.2) ‡	0.58 (0.11)	1.09 (0.16) ‡	0.75 (0.1)	1.40 (0.17) ‡	0.92 (0.29)	1.71 (0.28) ‡	1.11 (0.17)	2.19 (0.39) *	1.36 (0.24)

A, Achondroplasia; C, control; LTO, Left Toe Off; RHC, Right Heel Contact; $\boldsymbol{\lambda}$, dimensionless stride length; f, dimensionless stride frequency; Fr, Frounde's number; * P < 0.05, † P < 0.01, ‡ P < 0.001

Table 7.7: Average joint positions (°) of the pelvis, hip, knee and ankle in the sagittal plane only during incremental running speeds ($m \cdot s^{-1}$) in the group with Achondroplasia and controls. Values given as mean (SD).

	ME	1.67	1.96	2.22	2.5	2.78	3.06	3.33
Pelvis	P < 0.001	5.0 (3.4) ‡	3.9 (4.3) ‡	5.4 (3.3) ‡	4.0 (2.4) ‡	4.5 (3.0) ‡	2.7 (2.8) ‡	5.0 (3.7) ‡
Hip	P = 0.776	2.6 (2.4)	2.8 (2.1)	1.0 (1.7)	0.6 (1.8)	1.6 (2.9)	-1.6 (2.5)	-2.6 (3.6)
Knee	P = 0.082	6.0 (4.8)	6.3 (5.2)	6.2 (4.3)	4.6 (4.9)	6.9 (4.3)	5.1 (5.8)	3.0 (5.2)
Ankle	P = 0.014	5.1 (0.8) †	4.9 (1.1) *	3.7 (0.9) ‡	3.8 (0.8) *	5.0 (1.1) *	2.9 (1.0)	2.5 (1.5) *

ME, Main effect; * P < 0.05, † P < 0.01, ‡ P < 0.001

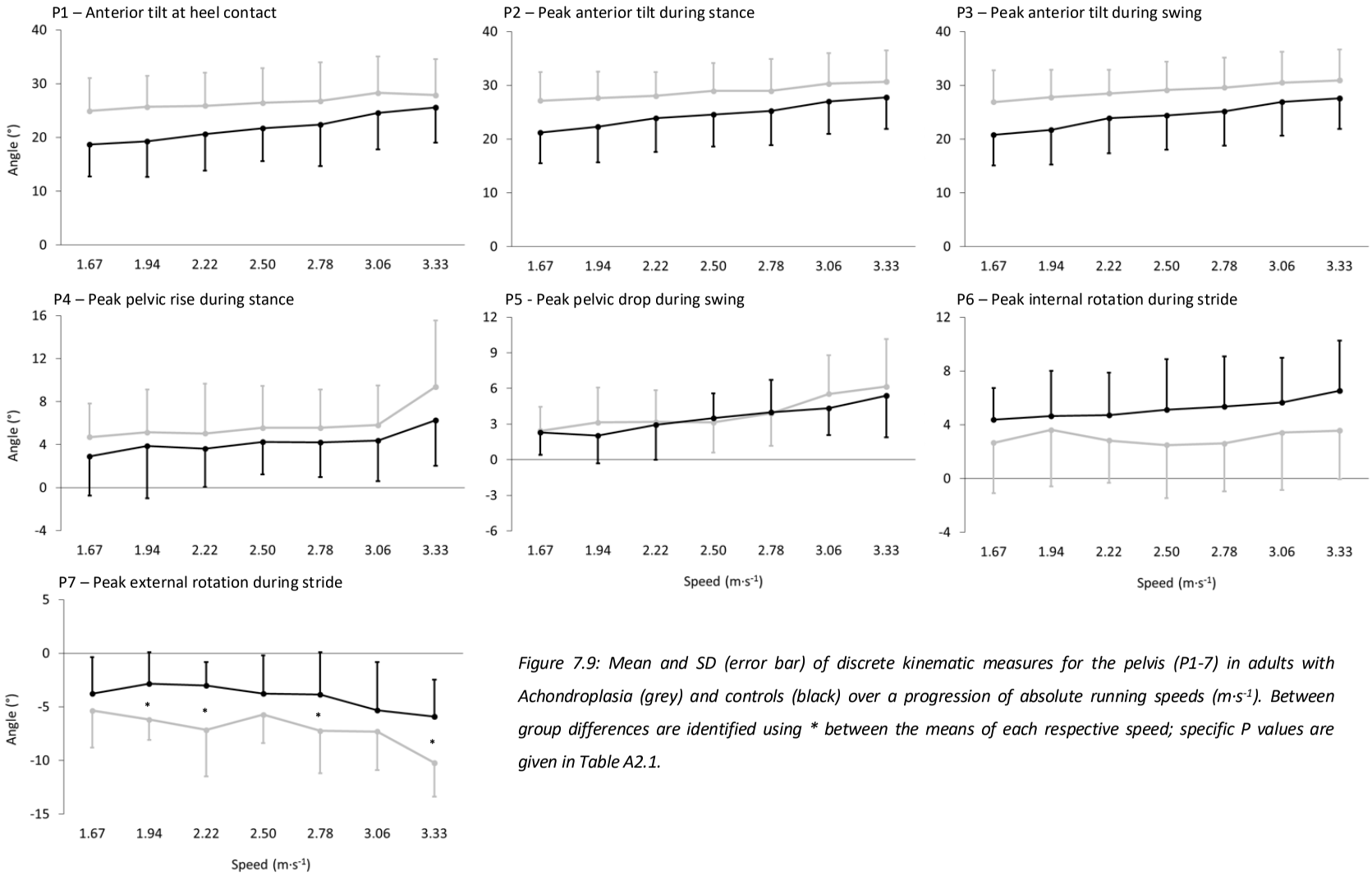

*Figure 7.9: Mean and SD (error bar) of discrete kinematic measures for the pelvis (P1-7) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute running speeds (m·s⁻¹). Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A2.1.*

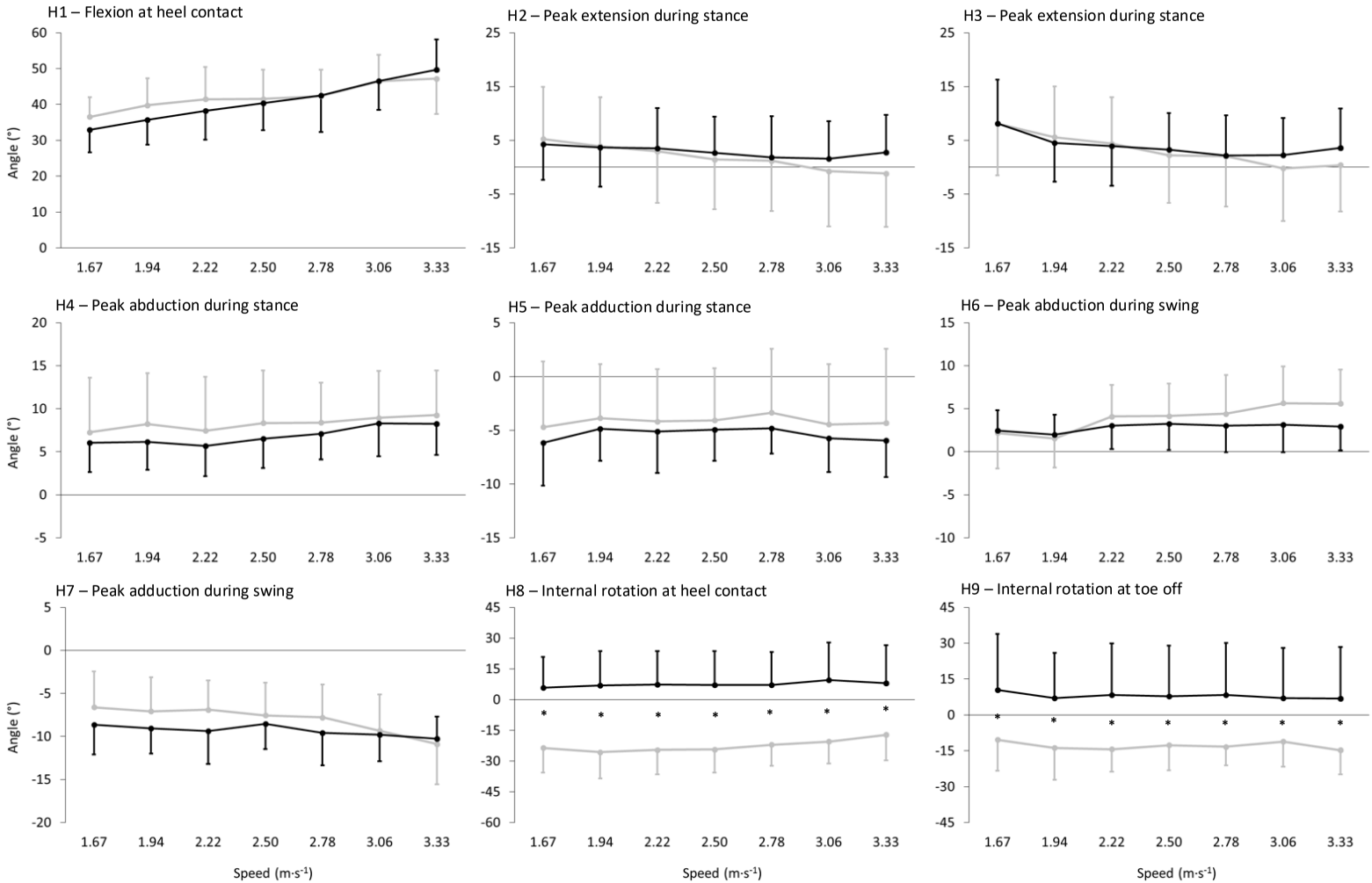

*Figure 7.10: Mean and SD (error bar) of discrete kinematic measures for the hip (H1-9) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute running speeds. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A2.2.*

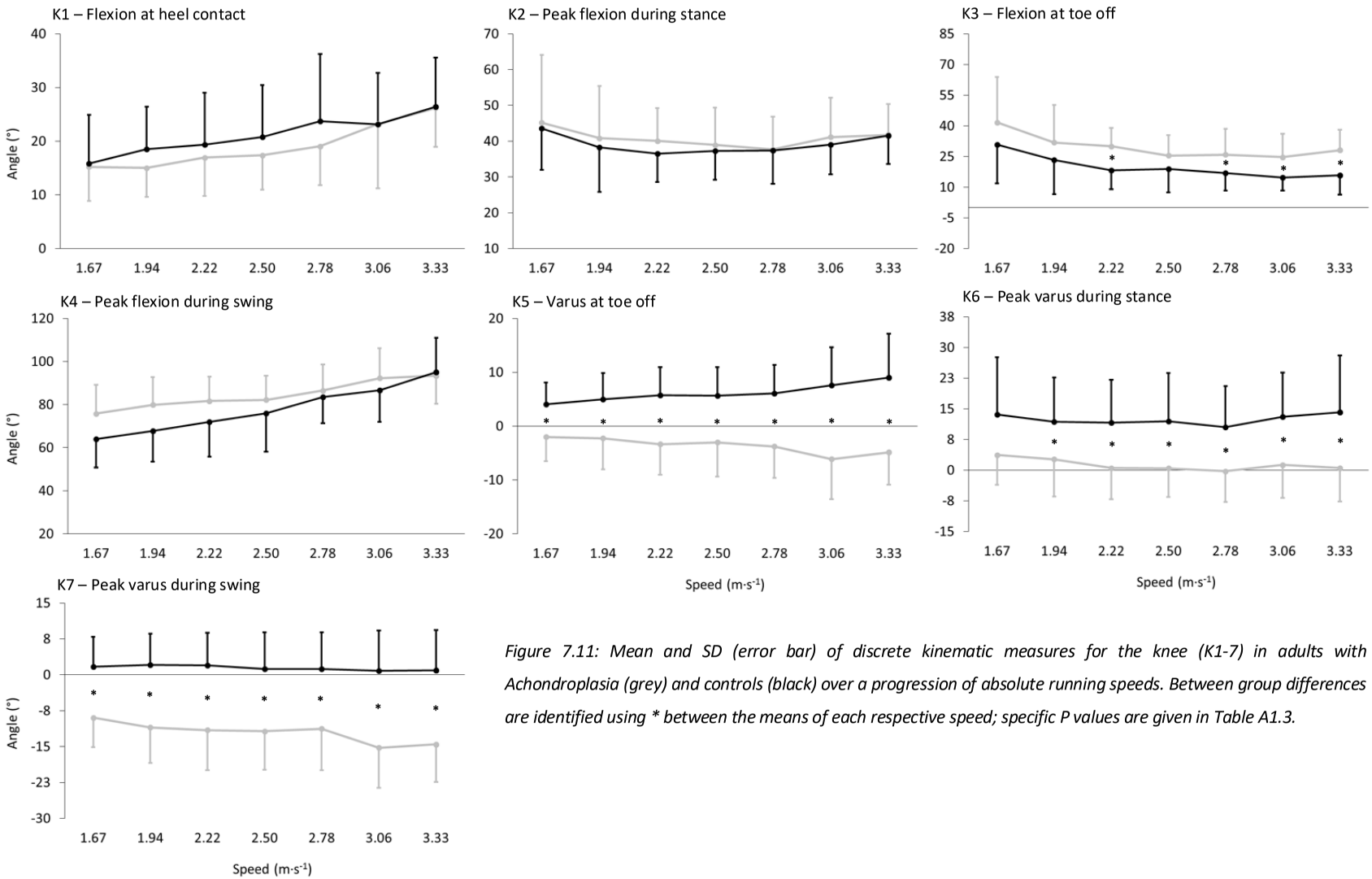

*Figure 7.11: Mean and SD (error bar) of discrete kinematic measures for the knee (K1-7) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute running speeds. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A1.3.*

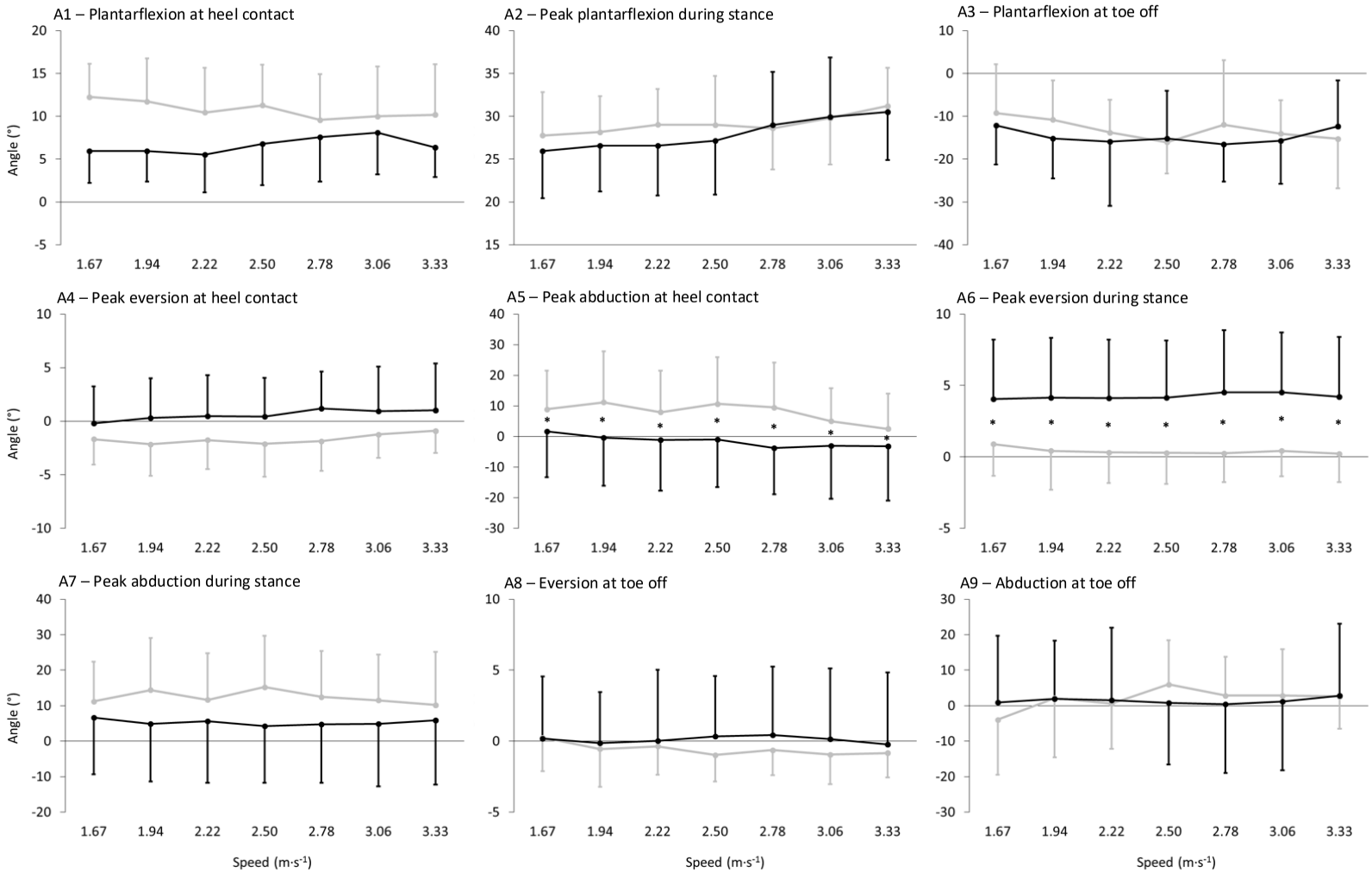

*Figure 7.12: Mean and SD (error bar) of discrete kinematic measures for the ankle (A1-9) in adults with Achondroplasia (grey) and controls (black) over a progression of absolute running speeds. Between group differences are identified using * between the means of each respective speed; specific P values are given in Table A2.4.*

Table 7.8: Absolute vertical displacement of the body's centre of mass from initial heel contact during incremental running speeds ($m \cdot s^{-1}$) in the group with Achondroplasia and controls. Values shown as mean (SD).

	1.67		1.94		2.22		2.50		2.78		3.06		3.33	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
Heel Contact (m)	0.74 * (0.02)	1.03 (0.04)	0.74 * (0.01)	1.03 (0.05)	0.73 * (0.01)	1.03 (0.05)	0.73 * (0.01)	1.03 (0.05)	0.73 * (0.02)	1.01 (0.05)	0.72 * (0.03)	1.01 (0.04)	0.72 * (0.01)	1.00 (0.05)
Min Height During Left Stance (m)	0.71 * (0.02)	0.98 (0.05)	0.71 * (0.02)	0.98 (0.05)	0.70 * (0.01)	0.98 (0.05)	0.70 * (0.01)	0.98 (0.05)	0.71 * (0.01)	0.97 (0.05)	0.70 * (0.03)	0.97 (0.05)	0.70 * (0.01)	0.96 (0.04)
Max Height During 1st Flight (m)	0.76 * (0.02)	1.06 (0.05)	0.76 * (0.02)	1.06 (0.05)	0.76 * (0.02)	1.06 (0.05)	0.75 * (0.02)	1.06 (0.05)	0.75 * (0.02)	1.04 (0.05)	0.74 * (0.03)	1.05 (0.05)	0.74 * (0.02)	1.04 (0.04)
Min Height During Right Stance (m)	0.70 * (0.02)	0.98 (0.05)	0.70 * (0.02)	0.98 (0.05)	0.70 * (0.01)	0.98 (0.05)	0.70 * (0.01)	0.97 (0.05)	0.70 * (0.01)	0.97 (0.05)	0.70 * (0.03)	0.97 (0.04)	0.70 * (0.01)	0.97 (0.04)
Max Height During 2nd Flight (m)	0.76 * (0.02)	1.07 (0.05)	0.76 * (0.02)	1.07 (0.05)	0.76 * (0.02)	1.07 (0.05)	0.76 * (0.02)	1.06 (0.05)	0.75 * (0.02)	1.06 (0.05)	0.75 * (0.03)	1.06 (0.05)	0.75 * (0.02)	1.06 (0.04)

A, Achondroplasia; C, Control; Differences between group for same speed only; ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.

Table 7.9: Absolute vertical displacement of the body's centre of mass from initial heel contact during incremental running speeds ($m \cdot s^{-1}$) in the group with Achondroplasia and controls. Values shown as mean (SD).

	1.67		1.94		2.22		2.50		2.78		3.06		3.33	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
Total left-to-right displacement (m)	0.09 † (0.02)	0.08 (0.03)	0.09 † (0.03)	0.08 (0.03)	0.08 † (0.02)	0.06 (0.03)	0.08 * (0.02)	0.05 (0.02)	0.07 * (0.02)	0.06 (0.02)	0.08 † (0.02)	0.06 (0.03)	0.09 * (0.02)	0.07 (0.02)
Min Height During Left Stance (m)	-0.03 * (0.01)	-0.05 (0.01)	-0.03 * (0.01)	-0.05 (0.01)	-0.03 † (0.01)	-0.05 (0.01)	-0.03 * (0.01)	-0.05 (0.01)	-0.03 † (0.01)	-0.04 (0.01)	-0.02 * (0.01)	-0.04 (0.01)	-0.02 * (0)	-0.04 (0.01)
Max Height During 1st Flight (m)	0.01 † (0.01)	0.03 (0.01)	0.02 ‡ (0.01)	0.03 (0.01)	0.03 (0.02)	0.03 (0.02)	0.02 (0.01)	0.03 (0.02)	0.01 * (0.01)	0.03 (0.02)	0.03 (0.01)	0.04 (0.02)	0.02 ‡ (0.02)	0.04 (0.01)
Min Height During Right Stance (m)	-0.04 (0.01)	-0.05 (0.02)	-0.04 ‡ (0.01)	-0.05 (0.02)	-0.04 † (0.01)	-0.05 (0.01)	-0.03 * (0.01)	-0.05 (0.01)	-0.03 (0.01)	-0.04 (0.02)	-0.02 † (0.01)	-0.04 (0.02)	-0.02 (0.01)	-0.03 (0.02)
Max Height During 2nd Flight (m)	0.02 † (0.01)	0.03 (0.02)	0.02 † (0.01)	0.04 (0.02)	0.02 † (0.02)	0.04 (0.01)	0.03 ‡ (0.01)	0.04 (0.01)	0.02 * (0.01)	0.05 (0.02)	0.03 † (0.01)	0.05 (0.02)	0.03 * (0.02)	0.06 (0.02)

A, Achondroplasia; C, Control; Differences between group for same speed only; ‡ $P \leq 0.05$, † $P \leq 0.01$, * $P \leq 0.001$.

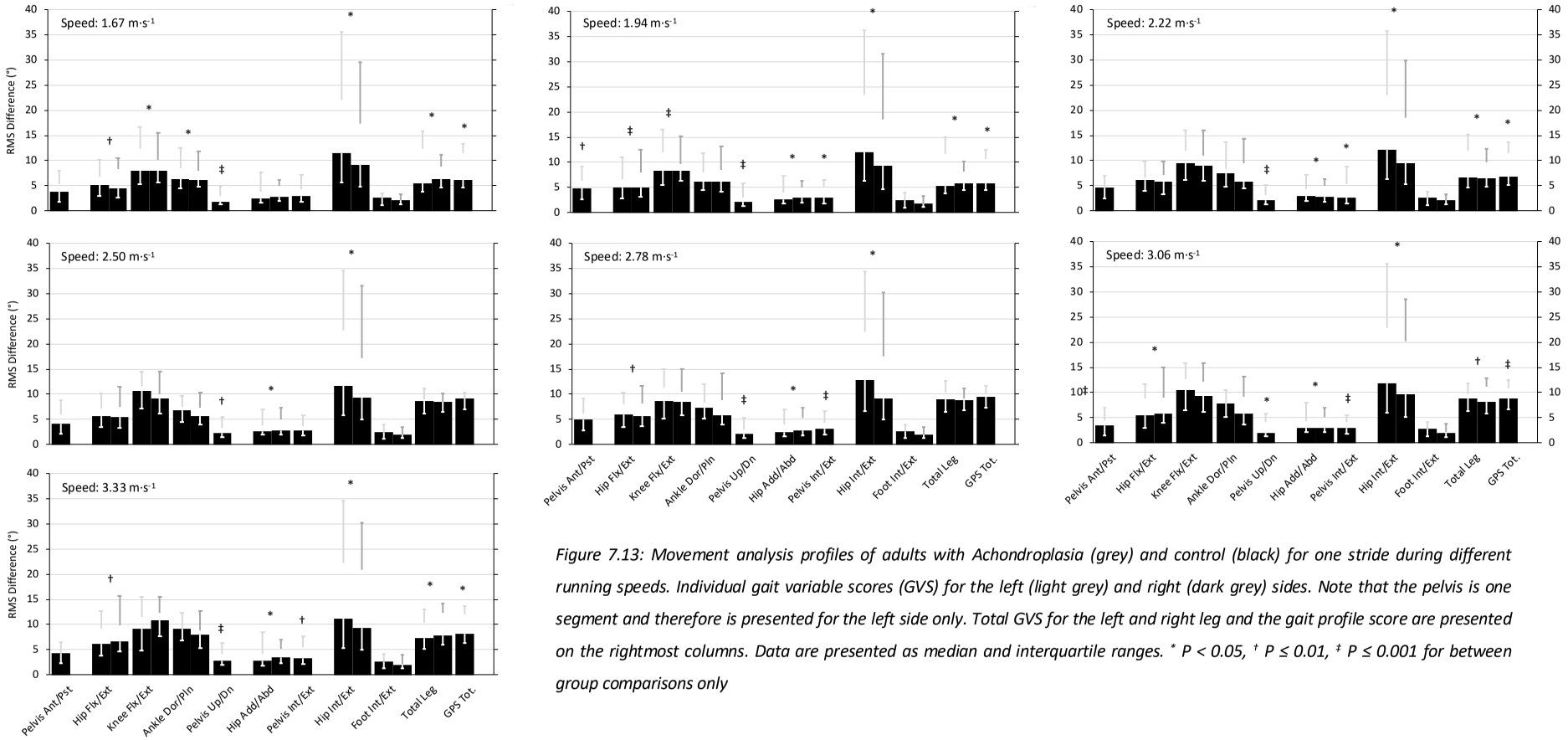

*Figure 7.13: Movement analysis profiles of adults with Achondroplasia (grey) and control (black) for one stride during different running speeds. Individual gait variable scores (GVS) for the left (light grey) and right (dark grey) sides. Note that the pelvis is one segment and therefore is presented for the left side only. Total GVS for the left and right leg and the gait profile score are presented on the rightmost columns. Data are presented as median and interquartile ranges. * P < 0.05, † P ≤ 0.01, ‡ P ≤ 0.001 for between group comparisons only*

Part 4: General kinematic discussion

7.6.p4.1 Discussion

This study aimed to measure spatio-temporal parameters, joint kinematics of the lower limbs, and CoM translations during incremental walking and running in adult males with Achondroplasia without limb lengthening surgery and controls. The main findings were that: 1) the group with Achondroplasia had a higher stride frequency and shorter stride length than controls at every walking and running speed; 2) NDN stride frequency and stride length did not eliminate the spatio-temporal differences between groups; 3) the group with Achondroplasia appear more 'flexed' at the pelvis, hip, knee and ankle throughout the stride when walking, but only at the pelvis and ankle when running, compared to controls; 4) as a global measure (GPS), the gait of individuals with Achondroplasia is quantifiably different to controls at every walking speed with differences lessening between groups when running; and 5) relative to initial position at heel contact, vertical displacement of the CoM was lower in the group with Achondroplasia at every gait speed, whereas their medio-lateral CoM movement was larger compared to controls at some walking speeds. With limited gait related research in populations with Achondroplasia (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008), this section of the discussion will focus on the difference in variables between groups' SSW speed.

With stride length being determined somewhat by leg length (Hof, 1996) and the group with Achondroplasia having shorter legs than controls (Chapter 2), their shorter stride length and higher stride frequency than controls at each set speed is

not surprising. More surprisingly is that all temporal gait events were similar between groups when normalised to stride time. Despite these events being the same between groups when time normalised, only stride frequency was similar between groups at set walking and running speeds when presented as NDN values. The NDN of spatio-temporal measures at SSW are commonly used within the literature to help to describe the natural oscillation of the legs (Minetti et al., 2000; Vaughan and O'Malley, 2005; P. A. Kramer and Sylvester, 2012). The higher *Fr* and NDN stride lengths in the group with Achondroplasia across all speeds suggests that they are taking relatively longer steps and translating relatively quicker than controls at set speeds. These differences are likely due to changes in kinematics, brought about to maintain the most economical stride frequency at set speeds (Minetti et al., 1995), and discussed in more depth in section 8.5 of Chapter 8.

The group with Achondroplasia had a slower SSW speed than controls and a higher absolute stride frequency, which is consistent with other comparisons of shorter versus taller groups, such as children (Stolze et al., 1997; P. A Kramer and Sarton-Miller, 2008). When SSW spatio-temporal variables were presented as NDN values, the two groups in this study had a similar *Fr*, but the group with Achondroplasia had a longer NDN stride length and lower stride frequency than controls. A similar *Fr* indicate that group's relative speed is similar to another and therefore the two groups move in a dynamically similar way (Minetti et al., 2000); although the differences in NDN stride length and frequency between the groups suggest otherwise. The kinematic patterns of gait in the group with Achondroplasia (Figure A1 and A2, Appendix 1) suggest that their relatively longer stride length may be

misleading. Despite the more flexed position in the group with Achondroplasia throughout the gait cycle compared to controls, the positions of the pelvis, hip and knee at heel contact are the same between groups. The range of motion of the joints is also similar from initial heel contact to second heel contact (Figure A1, Appendix 1), suggesting that the relative stride length should be the same between groups. Despite the differences in NDN spatio-temporal values between groups, the inclusion of such parameters are used to help explain variability in physiological or biomechanical variables, such as C or joint power (Hof, 1996; Moisio et al., 2003; Pinzone et al., 2016; Chia and Sangeux, 2017). The differences in NDN values between the presented groups may not, therefore, be explained by kinematic differences, and could be due to differences in inertial parameters (e.g. moment of inertia and radius of gyration positions) between groups. The NDN values collected here, are more likely to help explain variability in physiological and/or biomechanical variables between the group with Achondroplasia and controls, as done in Chapter 4 (relationship between $\dot{V}O_2$ and *Fr*).

The group with Achondroplasia are indeed more flexed at all speeds when walking and running, which is similar to previous reports of the population (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008). For example, the SSW sagittal plane kinematic patterns in the current group with Achondroplasia appear to be more flexed than those reported by van der Meulen et al. (2008). van der Meulen et al. (2008) reported on a population with Achondroplasia with demographics similar to the current study (N = 11; age 24 (6) yrs), but their study's population had undergone leg lengthening surgery, which the

current group had not. Increasing leg length while maintaining natural foot length, as reported by van der Meulen et al. (2008), would result in a shorter foot-to-leg length ratio. This would require less knee flexion and less dorsiflexion during the swing phase to avoid toe contact with the floor and in turn lower the risk of falling (Mills et al., 2008). While this is a valid theory, contradictory data exist in a non-leg lengthened groups with Achondroplasia who exhibit less knee flexion during the stride compared to the current group with Achondroplasia (Egginton et al., 2006). This discrepancy is likely due to a more flexed hip angle during swing in Egginton's group with Achondroplasia, reducing the need for increased knee flexion during swing phase to avoid toe contact with the floor. Differences in hip and knee flexion between the current group with Achondroplasia and Egginton's group may be due to differences in the hip joint centre prediction used in the respective gait models and their appropriateness for control populations, as discussed in section 8.4.1 in Chapter 8.

There are also more substantial differences in the frontal plane kinematics of the knee between the present study and available gait kinematic data in populations with Achondroplasia. In the present study, the knee of the group with Achondroplasia is more valgus than the controls' during stance. This is possibly explained by either their more compliant patella tendon (and hypothesised compliant joint) than controls (as exhibited in Chapter 6), or, differences in joint centre predictions from the kinematic model used (discussed in section 8.4.1 of Chapter 8). For example, based on observations in Chapter 6 and assuming the relative ground reaction force (N ÷ body weight) is the same between groups, the

knee of individuals with Achondroplasia would displace more during stance than controls leading to a more valgus position; this is dependent on the vector of the ground reaction force. While this may explain frontal knee kinematic differences between groups, there are appears to be no joint laxity measures made during gait in populations with Achondroplasia, nor are there any specific gait models available to provide a more valid description of joint kinematics in the population. The knee of the current group with Achondroplasia remains in a more neutral position, although valgus, compared to children with Achondroplasia; they exhibit a varus knee position during the stance phase (Inan et al., 2006). Tendon compliance is lower in child controls compared to adult controls, with a more lax joint displacing more under load (O'Brien et al., 2010b). These differences seen in controls are likely to be consistent in populations with Achondroplasia and may explain the more varus position knee during stance in children with Achondroplasia compared to controls (Inan et al., 2006).

The different joint positions during gait between groups may also explain the differences in vertical and medio-lateral displacement of CoM at all speeds between groups. For example, at ~25% of the gait cycle when walking, where the CoM is at its highest point for both groups, the group with Achondroplasia have a more flexed knee and dorsiflexed ankle. Being in this more flexed position leads to smaller vertical movements of the CoM relative to its initial position at heel contact. The group with Achondroplasia also had a more internally rotated hip and a more valgus knee than controls during the entire stride. The position of the thigh and shank during stance may explain some of the difference between the groups' CoM medio-

lateral translation during some walking speeds. Similar observations of CoM movement are made in clinical populations with affected gait (van den Hecke et al., 2007; Weinert-Aplin et al., 2017), shorter stature (Minetti et al., 1994; Minetti et al., 2000) and obese groups (Browning and Kram, 2007). The combined vertical and medio-lateral movement of the CoM is likely to have an effect on the external work done by the group with Achondroplasia which will have implications on the C of their gait (Cavagna et al., 1983) (see Chapter 4 and section 8.5 of Chapter 8). The altered walking kinematics of the group with Achondroplasia, which are possibly brought about by the need to avoid toe contact with the floor during the swing phase, may not only affect the CoM translations, but also the calculated GPS.

The group with Achondroplasia had a consistently higher GPS compared to controls when walking and running. With GPS consisting of 15 joint kinematics, persistent differences in joint angle during the stride, including more flexed joint positions, will be highlighted as a larger GPS. For example, the knee and ankle at SSW have a 55 and 64% higher flexion/extension and plantar/dorsiflexion GVS than controls, respectively, which contributes to the overall higher GPS. The SSW GPS of the group with Achondroplasia (11.4° (2.0)), is consistent with conditions that present musculoskeletal impairments such as spina bifida, ligamentous laxity, paraplegia and Cerebral Palsy (7.5 - 14.5° (Baker et al., 2012; Schweizer et al., 2014)). As the group with Achondroplasia have a more flexed lower limb, the use of GPS to compare their gait to controls may be viewed with caution. It would be more appropriate to compare the GPS of a population with Achondroplasia to other populations with

Achondroplasia (e.g. natural leg length vs leg lengthened) to observe clinical relevance.

7.6.p4.2 Walking vs running

When running, the group with Achondroplasia had more flexed lower limbs than the controls, but to a lesser extent than when walking. What appears as a more similar gait between groups when running is likely due to the inclusion of a flight phase. With the group with Achondroplasia having a longer foot-to-leg length than controls (Chapter 2), they need to flex their knee and/or dorsiflex their ankle more during swing to avoid toe contact with the floor; this is assuming that other joint positions are similar between groups throughout the gait cycle. For example, were the groups' hip and ankle in a similar position throughout the gait cycle, the group with Achondroplasia would need to flex their knee more during gait to avoid toe contact. Alternatively, were the hip and knee of both groups to remain in a similar position throughout the gait cycle, the group with Achondroplasia would need to dorsiflex more through the swing phase to avoid contact with the floor. Both, or either, scenario would contribute to the group with Achondroplasia being in a more flexed position during gait than controls, as observed in the present Chapter.

The toe is therefore higher from the floor when running (due to the body becoming a projectile) than when walking which means that the hip and knee and ankle do not need to flex and dorsiflex, respectively, as much to avoid toe contact during the swing phase. This was measured in this Chapter as similar hip and knee joint positions

between groups when running, but the group with Achondroplasia have a more dorsiflexed ankle than controls. This is also acknowledged in discrete sagittal joint kinematics, as the group with Achondroplasia had more knee flexion than controls at toe off when running only. Whereas when walking, the group with Achondroplasia had more peak knee flexion during the stance and swing than controls at all speeds. The measurement of joint kinematics during walking and running provide a hypothesis that individuals with Achondroplasia may present a more flexed gait when walking to avoid fall incidence. The multi-speed kinematic analysis of gait presented in this Chapter appears to be the only such data in any population with Achondroplasia, further work is required to help support this.

7.7.p4.1 Conclusion

The current study aimed to present a comprehensive analysis of gait during walking and running in a homogenous adult population with Achondroplasia. The SSW speed of individuals with Achondroplasia is slower and their stride frequency is greater than controls, whilst relative stride durations are similar between groups at all walking and running speeds. Numerous differences in discrete kinematics of the lower limbs exist between the groups during walking and running, which combine to present a more 'flexed' and quantifiably different gait of individuals with Achondroplasia compared to controls. The differences in gait are possibly due to enable individuals with Achondroplasia avoid toe contact with the floor during the swing phase.

Chapter 8: General discussion

8.1 Introduction

There is a wealth of data surrounding the genetic mutation, fibroblast growth factor receptor 3 (FGFR3), and interventions related to increasing stature, such as growth hormone therapy (Horton et al., 1992) and surgical procedures (Park et al., 2015) for individuals with Achondroplasia. As established through the review of literature (Chapter 1), there is a distinct lack of functional outcome measures described in adults with Achondroplasia, despite descriptions of recovery from limb lengthening surgery. A description of primary outcome measures, such as oxygen consumption ($\dot{V}O_2$), maximal strength and gait performance, is essential to the understanding of any impairments associated with the individuals with Achondroplasia to then inform on stature increasing interventions. The primary aim of this thesis was to present physiological and biomechanical data related to functional tasks in an adult population with Achondroplasia. More specifically, this thesis addressed the following aims:

1) To describe accurately the *in vivo* mass distribution, body composition and anthropometry of adults with Achondroplasia;
2) To ascertain the maximal aerobic capacity ($\dot{V}O_{2max}$) of adults with Achondroplasia;
3) To describe the submaximal $\dot{V}O_2$ profile and metabolic cost (C) during incremental walking and running in adults with Achondroplasia;
4) To measure the *in vivo* isometric maximal voluntary contraction (iMVC) during knee extension in adults with Achondroplasia;

5) To determine the *in vivo* mechanical properties of the patella tendon of during knee extension iMVC in adults with Achondroplasia;
6) To describe the lower limb kinematics during walking and running of adults with Achondroplasia;

Throughout the thesis, data was presented relative to body dimension and body mass to enable more valid comparisons to age matched males without Achondroplasia (controls).

8.2 Main findings of the thesis

Adults with Achondroplasia have less bone mineral content and density (BMC and BMD respectively), less fat free mass (FFM) and more fat mass than controls at the total-body level with differences lessening when relative to total-limb values (Chapter 2); the $\dot{V}O_{2max}$ of adults with Achondroplasia is lower than controls, but similar when presented relative to total-body mass (TBM) (Chapter 3); walking and running C is higher in adults with Achondroplasia than controls, which persists when presented relative to TBM, fat free mass (FFM) and leg length (Chapter 4); absolute and relative iMVC force production of knee extensors and knee flexors, and specific force of the vastus lateralis (VL) is lower in adults with Achondroplasia compared to controls (Chapter 5); patella tendon Young's Modulus is lower in adults with Achondroplasia compared to controls (Chapter 6), and; the gait of individuals with Achondroplasia is more 'flexed' and quantifiably different to controls when walking and running (Chapter 7). Figure 1 shows a flow diagram of the main findings of each chapter and how they may interact with each other.

Two main themes were identified from the current thesis. Firstly, there some functional measures between groups that can be explained by body proportions, lengths, total-body masses and segmental-body masses (see section 8.3 below). When comparing measures of bone (Chapter 2), muscle (Chapter 5), tendon (Chapter 6) and gait (Chapter 7), differences between groups persisted when presented relative to relevant physiological and biomechanical measures. The differences in intrinsic bone properties between groups may be due to the mutated FGFR3 gene that causes Achondroplasia. Briefly, mutated FGFR3 amplifies the proliferation and differentiation of growth plate chondrocytes, stunting bone growth (Deng et al., 1996). Being a collagen defect, the mutation may also be a cause of the intrinsic differences of muscle (Chapter 5) and tendon (Chapter 6) but is beyond the scope of this thesis.

Secondly, the gait of individuals with Achondroplasia is quantifiably more 'flexed' than controls (see section 8.4 below). Gait differences may be due to differences in body proportions (Chapter 2), neuromuscular function (Chapters 5 and 6) and/or limitations to the measurement of gait kinematics (see section 8.4.1 below). The absolute and relative differences between groups' physiological and kinetic measures during iMVC and gait may have implications C (section 8.5 below) and therefore help explain the differences in C between groups (Chapter 4). This Chapter will discuss the main themes from this thesis and link the respective findings from each experimental Chapter in an attempt to explain the persistent difference in C between groups.

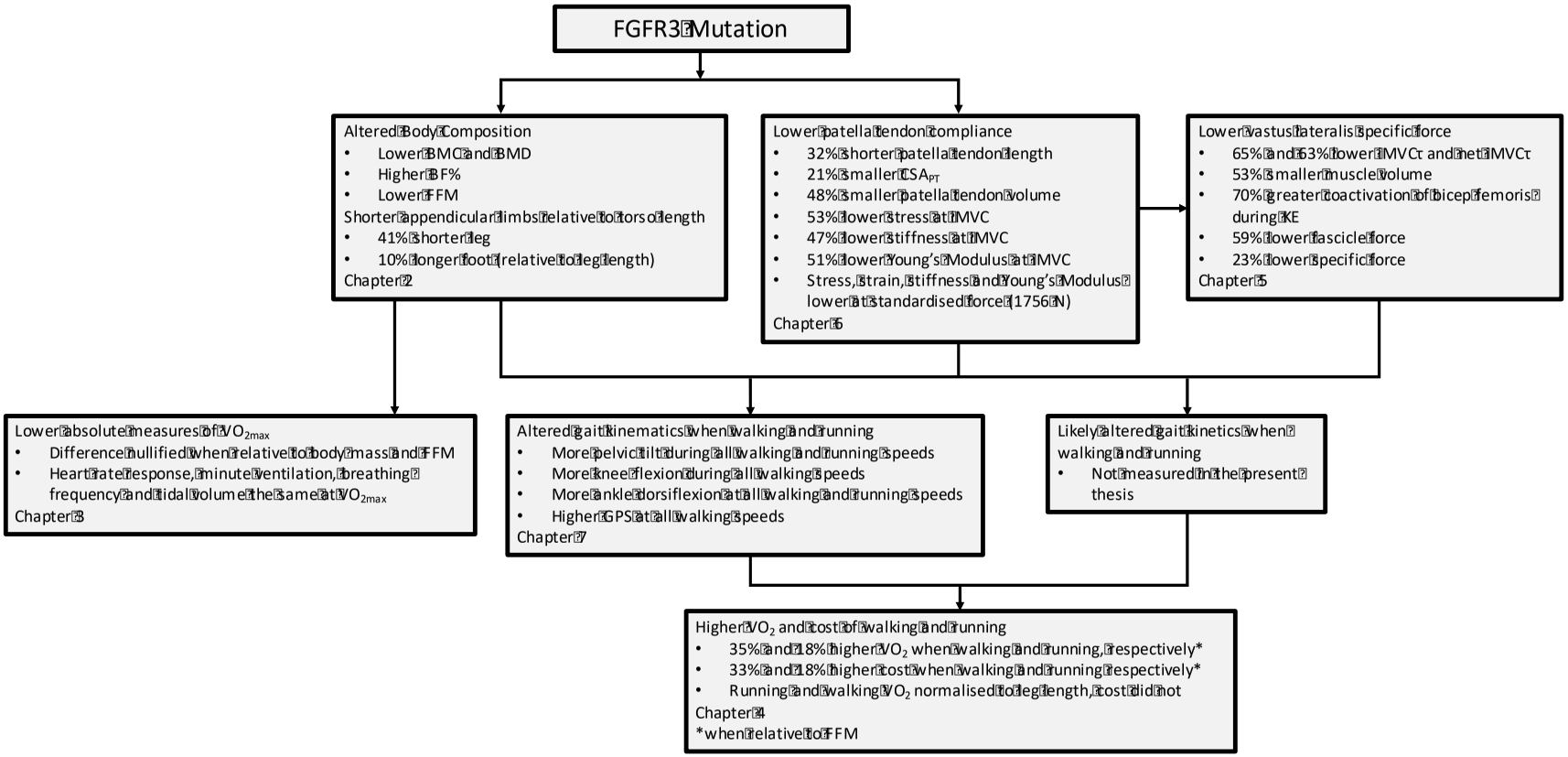

Figure 8.1: A flow diagram showing the main findings of each of the present thesis' chapters that are possibly manifested from the mutated fibroblast growth factor receptor 3 gene that causes Achondroplasia. All main findings are significant ($P \leq 0.05$) unless stated. Abbreviations: FGFR3, fibroblast growth factor receptor 3; BMC, bone mineral content; BMD, bone mineral density; BF%, body fat percentage; FFM, fat free mass; iMVCτ, isometric maximal voluntary contraction torque; KE, knee extension, CSA_{PT}, cross sectional area of the patella tendon; iMVC, isometric maximal voluntary contraction; $\dot{V}O_{2max}$, maximal oxygen consumption; GPS, gait profile score; $\dot{V}O_2$, oxygen consumption.

8.3 Are individuals with Achondroplasia disproportionate?

This thesis aimed to measure numerous neuromuscular and kinematic variables during maximal and submaximal functional tasks in adults with Achondroplasia. Where differences were observed in physiological and biomechanical variables, these were presented relative to body dimensions and total-body and segmental mass, due to the disproportionate dimensions of the body of individuals with Achondroplasia (i.e. limb-to-torso lengths relative to controls). Based on the available data, and those presented in Chapter 2, the group with Achondroplasia in this thesis are osteopenic using the current classification (1.5 SD lower than controls, French et al. (2002)), but when BMD was presented relative to total-limb BMD and volumetric BMD (Chapter 2), no differences were observed between groups. In fact, the group with Achondroplasia had higher BMD of the shank when expressed relative to total-limb measures. The absolute $\dot{V}O_{2max}$ was lower in the group with Achondroplasia whereas submaximal $\dot{V}O_2$ was higher than controls. When $\dot{V}O_{2max}$ was presented relative to TBM and FFM, and $\dot{V}O_2$ to TBM and geometric measures (Froude's number, *Fr*) however, differences between groups were accounted for (Chapter 3 and 4, respectively). Surprisingly, a lower specific force of the VL was observed in the group with Achondroplasia compared to controls' (Chapter 5); this result however, may have been due to methodological limitations and are discussed later in this Chapter (section 8.6). In Chapter 6, morphological and dimensional measures of the patella tendon were accounted for during loading (stress ÷ strain = Young's Modulus). Despite this, the patella tendon in the group with Achondroplasia was more compliant than controls'. Indeed, whilst the group with Achondroplasia are anatomically and morphologically disproportionate to controls, this thesis has

shown that some clinical measures and exercise tasks can be scaled to body dimensions and masses, and are thus comparable to controls from a qualitative perspective.

Furthermore, as with other populations where morphological differences describe functional differences (e.g. children, sex, stature), it is apparent that meaningful physiological differences in individuals with Achondroplasia are largely negated when the appropriate morphological differences are considered. For example, total-body measures suggest that groups with Achondroplasia are at risk of a number of health complications (e.g. osteopenia and increased risk of cardiovascular events) when compared to control reference data (French et al., 2002; T. Kelly et al., 2009). When these data are expressed relative to total-body and total-limb values however, this may not be the case. The commonly used normative data sets to define health states of control populations are valid given the similarity in the populations used to provide such data (T. Kelly et al., 2009). However, comparisons are only valid when they are between populations of similar age, sex and stature, of which there are ample data for control populations. In Chapter 2 it was demonstrated that, due to the disproportionate torso-to-limb length ratios between groups, such normative data sets (i.e. made up of large control database) are redundant for individuals with Achondroplasia. To the author's knowledge, there remains no extensive body composition database for specific comparisons between groups with Achondroplasia. Therefore, the data and analysis from the current study suggest that the classification of health states of individuals with Achondroplasia be made with

caution by clinicians or with an attempt to present segmental data relative to total-body or total-limb mass, as done in Chapter 2.

8.4 Why is the gait of individuals with Achondroplasia 'different'?

In Chapter 7 a number of different discrete gait kinematic variables were presented between the groups included in this thesis and to other groups with gait limiting pathologies. The comparison of the entire gait cycle between populations is complex though, mainly due to the number of kinematic variables biomechanical models can create. While the observations of joint kinematics of the group with Achondroplasia were consistent with other groups with Achondroplasia (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008), to help identify a global score of gait, the Gait Profile Score (GPS) was used. Results showed that the gait of the group with Achondroplasia was quantifiably different to controls.

The GPS was devised for, and used in the present thesis, to describe the gait cycle using a single number. GPS has been used to describe and determine gait 'quality' in clinical populations compared to controls (Baker et al., 2009; Beynon et al., 2010; Baker et al., 2012). The use of GPS alone may be useful as a clinical measure, but does not allow for comparisons of kinematic patterns and does not provide a full explanation for the differences between groups' GPS; this is expanded throughout this section and in section 8.4.1. The results presented in Chapter 7 suggest that the gait of individuals with Achondroplasia is different to controls' and to that of other musculoskeletal impaired conditions (self-selected walking (SSW) GPS of individuals

with Achondroplasia 11.4° (2.0); controls 4.1° (1.8); individuals with spina bifida, ligamentous laxity, paraplegia and Cerebral Palsy, 7.5 - 14.5° (Baker et al., 2012; Schweizer et al., 2014)).

The GPS is made up of 15 gait variable scores (GVSs). The largest differences between groups' GVSs lay in the sagittal plane (knee flexion/extension (55% at SSW) and ankle plantar/dorsiflexion (64% at SSW)) and the transverse plane (hip internal/external rotation (50% at SSW)). These are consistent with the differences in sagittal and transverse kinematic patterns and discrete gait events observed in the current thesis and elsewhere (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Inan et al., 2006; van der Meulen et al., 2008). While the difference in groups' GPS values are a result of these different gait kinematics, there are a number of potential reasons for the gait of individuals with Achondroplasia to exhibit a 'more flexed' position of lower limb joints and therefore a lower 'quality' of gait than controls. Some of the difference in the more flexed gait of individuals with Achondroplasia compared to controls is expanded on in Chapter 7 (section 7.5.1, which discusses the foot-to-leg length ratio and toe clearance during walking and running). The term 'quality', which has been associated with GPS, is ambiguous in the current context as it has limited biomechanical or physiological meaning. Certainly, a greater GPS infers a larger 'difference' in gait, but not necessarily a lower quality. For example, altering one's gait to maintain locomotion and reduce the likelihood of falls implies a high level of gait quality. For the groups with Achondroplasia, their apparent altered gait, which may be required to maintain locomotion, is measured as a higher GPS value compared to controls; this GPS value infers a lower quality of gait. This thesis

presents numerous variables that could elicit the subtle differences in gait between groups which in turn lead to a higher GPS value for individuals with Achondroplasia.

The lower force production of the VL during iMVC, and assumed lower force production of other ambulatory muscles, in individuals with Achondroplasia compared to controls (Chapter 5) are likely to be apparent during gait. A lower propulsive ground reaction force (GRF) during stance, as a result of the lower force generated from the legs, would not project the body as far as a higher GRFs. This would likely lead to a lower vertical movement of the body's centre of mass (CoM), relative to its position at heel contact, and a shorter stride length; both of which are observed in the group with Achondroplasia in Chapter 7. To maintain locomotion whilst the having a lower CoM, the leg would either displace through the swing phase quicker or not displace as far as; the latter was observed in the group with Achondroplasia compared to controls (Chapter 7). A more flexed hip, knee and ankle are therefore required to avoid tripping, again observed in the group with Achondroplasia (Chapter 7). Further to a lower specific force and more compliant tendon of the group with Achondroplasia at iMVC, a more compliant patella tendon was also observed at a standardised force (1756 N). This would suggest that the joints of individuals with more compliant tendons displace more during tasks that require the same force production (i.e. set gait speeds) compared to those with stiffer tendons. Assuming that other tendons that are involved in gait (such as the Achilles) are more compliant in individuals with Achondroplasia compared to controls, more joint displacement during concentric, eccentric and isometric loading of the ambulatory joints would be observed (Rosager et al., 2002). This in turn would

change the joint kinematics during gait and may explain some of the differences in certain joint kinematics between groups, such as the knee valgus/varus angles (Chapter 7).

While this thesis presents a number of neuromuscular differences that could alter gait kinematics, the contribution of the VL and patella tendon would only explain some of the knee joint kinematics and not the overall 'atypical' gait of the group with Achondroplasia. It is likely therefore, that the difference in gait between groups are due to either 1) the anthropometric differences between groups, such as the foot-to-leg length ratio, which requires an altered gait pattern to avoid toe contact with the floor, 2) morphological differences in joints associated with ambulation, of which there are limited data in groups with Achondroplasia (Akyol et al., 2015) or, 3) systematic differences in the model used to measure joint kinematics during gait.

8.4.1 Are the differences in gait between groups genuine or measurement error?

This thesis used the Plug-in-Gait model to predict lower extremity joint centres of both groups. Predicted joint positions were then used to estimate limb lengths (Chapter 2) and joint kinematics during walking and running (Chapter 7). Plug-in-Gait is a hierarchal model where distal joints are predicted based on proximal joints. For gait kinematics therefore, the accurate prediction of the hip joint centre (HJC) is of utmost importance. The HJC prediction in Plug-in-Gait is based on leg length and the anterior and posterior processes of the iliac spine. Whilst Plug-in-Gait does not use a direct HJC marker, an interpretation of the HJC (the greater trochanter) is required

to place the lateral thigh marker. Inaccurate placement (i.e. too anterior or posterior) of the thigh marker can lead to over- or under-predicted hip rotation and knee flexion angles during movement, known as “cross-talk” (Kadaba et al., 1990; Baker et al., 1999). A separate analysis of the HJC in the current group with Achondroplasia suggests that the Plug-in-Gait HJC may have inadvertently caused cross-talk. Figure 8.2 shows the average estimated HJC of Plug-in-Gait for both the group with Achondroplasia and controls during calibration and the estimated greater trochanter position (extrapolated line from knee and thigh markers) for the group with Achondroplasia only. The Plug-in-Gait HJC appears to be 32.5 mm posterior to the interpreted HJC from the greater trochanter; the thigh marker is then in a more anterior position in the Plug-in-Gait model. When using a 50 mm wand thigh marker (which this thesis did not use), for each 5 mm of anterior/posterior misplacement a ~3° additional internal/external hip rotation is observed (Baker, 2013). Assuming this is similar when using skin markers, ~20° of additional internal hip rotation would be observed in the current group with Achondroplasia. The difference in groups’ SSW knee valgus position at peak knee flexion during swing, where cross-talk is most likely to occur, is ~24° (Figure A1.1, Appendix 1). The apparent posterior HJC predicted by Plug-in-Gait in the group with Achondroplasia may therefore cause some of the greater knee flexion, internal hip rotation and knee valgus angles reported in the group, but there is little work into the HJC prediction of groups with Achondroplasia to confirm this.

What appears to be the only study to estimate the HJC of individuals with Achondroplasia shows contrary data to those presented in Figure 8.2. Broström et

al. (2009) showed that a functional method (Ehrig et al., 2006) identified the a 15.6 mm more posterior HJC in individuals with Achondroplasia (range 1.7 – 31.3 mm) than the Plug-in-Gait model (Davis et al., 1991). Broström et al. (2009) suggested that a functional prediction of HJC in individuals with Achondroplasia should be incorporated into future gait research. Were the functional model suggested by Broström et al. used in the present study, the HJC in the group with Achondroplasia would have been even more posterior than that predicted by Plug-in-Gait and only exacerbate the apparent cross-talk observed in Chapter 7. Were either the Plug-in-Gait and/or the model suggested by Broström et al. (2009) correct in identifying the HJC of individuals with Achondroplasia, the palpable identification of the greater trochanter would only misalign the thigh marker. This in turn would cause cross-talk and errors in hip internal/external rotation, again likely altering the kinematic patterns of individuals with Achondroplasia.

Regardless of other available models or suggestions for gait analysis in groups with Achondroplasia (Ehrig et al., 2006; Broström et al., 2009), the identified 32.5 mm more posterior HJC (relative to the greater trochanter) in the group (Chapter 7) would likely only alter knee flexion and internal hip rotation joint angles. With Plug-in-Gait being hierarchical, the ankle angle is based on the respective knee and ankle joint centres. Therefore, the more posteriorly tilted pelvis and more dorsiflexed ankle observed in the group with Achondroplasia throughout the walking and running stride can be interpreted as a more accurate joint position. The potential inaccurate prediction of the HJC in the group with Achondroplasia may therefore only explain some of their more 'flexed' position through the stride compared to controls. It does

not explain the more anteriorly tilted pelvis and dorsiflexed ankle of the group with Achondroplasia when walking and running compared to controls.

It is probable therefore, that individuals with Achondroplasia are indeed more 'flexed' through their gait cycle and, due to their more anteriorly tilted pelvis and dorsiflexed ankle through gait, their GPS would be higher than controls regardless of the model used. It is also probable that the potential error in joint kinematic measures of the group with Achondroplasia are systematic and the results from this thesis could be adjusted were the HJC prediction corrected for. Notwithstanding the above comments, this was beyond the scope of this thesis and is a basis for future work.

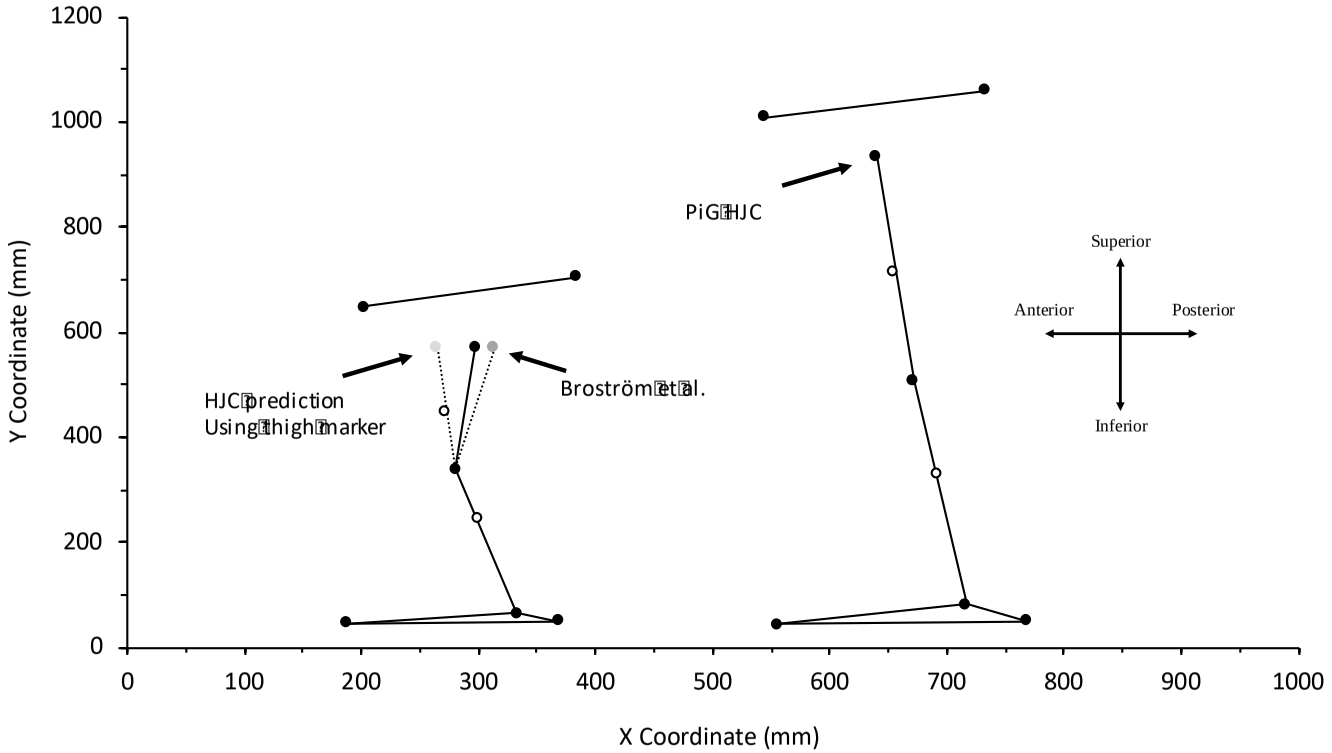

Figure 8.2: Sagittal view of the mean position Plug-in-Gait (PiG) marker for individuals with Achondroplasia (left) and control (right). Black circles represent the respective bony landmarks the hip joint centre (HJC) or the PiG model while white circles represents segment markers used for rotational variables. For the example of the group with Achondroplasia (left), the dark grey circles represent the estimated HJC from the equations presented by Broström et al. (2009) and the light grey circles represent the estimated HJC based on the palpable greater trochanter. Solid lines represent respective segments, from which joint angles are calculated and dotted line represents the estimated thigh segments based on the HJC prediction from Broström et al. (2009) and the palpable

8.5 Why is the metabolic cost of adults with Achondroplasia higher than controls?

Despite the use of body mass and leg length corrected speed (*Fr*), there was a higher C in the group with Achondroplasia compared to controls (Chapter 4). Walking and running C is a multifaceted parameter which combines biomechanical and physiological factors (Saunders et al., 2004). What was particularly interesting in Chapter 4 was that the average difference in walking C between groups (~20%) was higher than the average difference in running C (~12%). The current thesis observed a number of parameters that may explain the overall lower C in the group with Achondroplasia compared to controls and the similar C between groups when running compared to walking.

The lower specific force of the VL at iMVC in the group with Achondroplasia compared to controls suggests that for a given submaximal task, e.g. walking and/or running, the muscle requires greater activation to recruit the fibres needed to produce the same force required for that task, which is associated with greater C than in controls (Hortobágyi et al., 2011). Also, in Chapter 5, the group with Achondroplasia presented a greater coactivation of the hamstrings during iMVC knee extension than controls. This additional contraction of antagonists during knee extension is likely to increase $\dot{V}O_2$ of their gait, as observed in controls (Mian et al., 2006). However, both activation and coactivation profiles of locomotion muscles during gait were not measured in any of the Chapters within this thesis and is therefore scope for future work.

Further to lower VL force production than controls, the group with Achondroplasia also had a more compliant patella tendon at iMVC (Chapter 6). Given the more compliant patella tendon in the group with Achondroplasia, and assuming this finding is consistent in other tendons involved in locomotion, it is likely that there is an energy loss (through heat dissipation) during the lengthening of the tendons during with gait. The loss of energy, though hysteresis, was not measured in either group, but assuming there is greater hysteresis in the patella tendon of individuals with Achondroplasia (due to it being more compliant), the energy must be supplemented, which during exercise is identified by a higher C. This theory is observed in other controls groups, albeit predominantly in the Achilles tendon (Fletcher et al., 2010; Albracht and Arampatzis, 2013), and may explain some of the higher C during walking and running of the group with Achondroplasia. Assuming the lower force production and more compliant tendon contributes to the overall higher C in the group with Achondroplasia, these findings would not help explain the more similar C between the groups when running. It is therefore most likely that the difference in C between groups, and between modes of locomotion, is due to limb and CoM movement during gait.

Both limb movement and CoM translations are strongly linked with changes in C in controls, gait limited pathologies, shorter statured and clinical groups (Minetti et al., 1994; Minetti et al., 2002; Detrembleur et al., 2005; Browning et al., 2006; van den Hecke et al., 2007; Browning et al., 2009; Peyrot et al., 2009; Weinert-Aplin et al., 2017). van den Hecke et al. (2007) observed a higher external work, i.e. CoM movement relative to the environment, (Cavagna et al., 1964; Cavagna and Kaneko,

1977), due to greater medio-lateral movement of the CoM in individuals with Cerebral Palsy. Browning et al. (2007) observed similar results in the obese. Minetti et al. (1994; 2000) showed that African Pygmies and individuals with growth hormone deficient (GHD) populations do more internal work, i.e. movement of the limbs relative to the CoM, (Cavagna et al., 1964; Cavagna and Kaneko, 1977), when walking and running, but less external work when running compared to controls. The same African Pygmy and populations with GHD show a smaller difference in C compared to controls when running than when walking. Groups that exhibit a higher total mechanical work (internal + external work) invariably exhibit a higher C than controls (Ferretti et al., 1991; Minetti et al., 1994; Minetti et al., 2002; Detrembleur et al., 2005; Browning and Kram, 2007; van den Hecke et al., 2007; Peyrot et al., 2009). Based on the kinematic and GPS results from Chapter 7, it may be assumed that individuals with Achondroplasia moving their limbs more than controls, and therefore do more internal work during walking and running. The data in Chapter 7 also infers that individuals with Achondroplasia do less external work than controls when walking and running, due to their smaller relative vertical movements of the CoM. However, when walking, the group with Achondroplasia have greater medio-lateral movements than controls, which likely raises the amount of external work for walking and, in turn, leads to a higher C. While internal and external work were not directly measured here, differences in joint motions and CoM translations may explain the difference in C between groups and between modes of locomotion.

As described in Chapter 7 and above, the differences in gait of the group with Achondroplasia compared to controls may be required for them to maintain gait.

Therefore, possible differences in internal and external work are manifested by the necessity for a more flexed position. By incorporating measurements of internal and external work alongside metabolic measurements, calculation of walking and running efficiency could be estimated in populations with Achondroplasia. In groups of differing stature and mass, efficiency values during walking and running are similar to control populations (Ferretti et al., 1991; Minetti et al., 1994; Minetti et al., 2002; Griffin et al., 2003; Peyrot et al., 2009). Inclusion of internal and external work and C within a population with Achondroplasia may provide a better understanding of the relationship between a number of the variables measured in this thesis (i.e. force production, gait kinematics and energetics). To date, there appears to be no such measure in any population with Achondroplasia and is therefore a logical scope for future work in this population.

This thesis presents a multitude of physiological and biomechanical pathways that may explain the high C of adults with Achondroplasia compared to controls during running and, particularly, walking. However, only variables that may explain the higher C in isolation are presented, rather than during gait. Further work incorporating measures of tendon excursion and force production when analysing C throughout incremental speeds in both groups would further explain the data presented here. Furthermore, many of the methods used in this thesis were derived from, and validated in, control populations. The inclusion of such measures within the investigated group with Achondroplasia may have therefore incurred some inaccuracy in the results of this thesis.

8.6 Limitations to this thesis

As described in Section 2.3 of Chapter 2, the group with Achondroplasia included in this thesis represents the most homogenous skeletal dysplasic group available in the literature. While the data from this thesis are applicable to the other active males with Achondroplasia within the U.K. and the world, they do not necessarily represent other cohorts with Achondroplasia, such as females, the elderly, children or inactive males. There are certainly differences in $\dot{V}O_{2max}$, $\dot{V}O_2$, strength and tendon compliance between control populations of different ages, sexes and activity levels, which would suggest the same is apparent in other populations with Achondroplasia (Cuneo et al., 1991; Ferretti et al., 1991; Bottinelli et al., 1997; Goran et al., 2000; Reeves et al., 2003a; Tolfrey et al., 2006; Onambélé et al., 2007; Morse et al., 2008; O'Brien et al., 2010b; Hicks et al., 2017). Very few of the measures made here have been made in any populations with Achondroplasia, other than children (Rethlefsen and Tolo, 1998; Egginton et al., 2006; Takken et al., 2007; van der Meulen et al., 2008). What is likely however, is that in other skeletal dysplasic conditions, and the wider community with Achondroplasia, differences in functional measures to controls or between populations with Achondroplasia, such as strength and gait, are likely to be explained in part by morphological differences. What cannot be inferred from the present findings of this thesis, is by how much or by what magnitude even after scaling.

Chapter 5 and 6 showed a lower VL specific force and a more compliant patella tendon in individuals with Achondroplasia than controls, respectively. The measurement of both specific force and tendon compliance involved the respective

attainment of muscle volume using predetermined constants of magnetic resonance imaging (MRI) (Morse et al., 2007a) and patella tendon cross sectional area (CSA_{PT}) using ultrasound scans. Chapter 5 does describe the methodological limitation of using such estimations, but in brief, the denominator in the calculation of specific force appears to be a pseudohypertrophy, which then underestimates specific force. MRI would certainly improve the estimation of muscle volume within the group with Achondroplasia, but commercially available MRI analysis software, like B-mode ultrasound used in this thesis, does not consider fat infiltration within its estimate. The higher total-body fat content in the group with Achondroplasia compared to controls (Chapter 2) would likely be identified as muscle tissue rather than fat tissue when using the most basic function of either MRI or ultrasound. A pseudohypertrophy of the VL in the individuals with Achondroplasia may therefore have been observed, leading to a lower specific force compared to controls, as observed in Chapter 5. Without the insight of fibre biopsy derived measures of fat infiltration, the degree to which intramuscular adiposity contributes to the overestimation of muscle mass cannot be confirmed. Again, as described in Section 5.5.2 of Chapter 5, this limitation is not limited to individuals with Achondroplasia.

While ultrasound to quantify CSA_{PT} is considered reliable (Mc Auliffe et al., 2017) and has been used in numerous populations (Reeves et al., 2003a; Onambélé et al., 2007; K. E. Burgess et al., 2009a; Gellhorn and Carlson, 2013), the technique has not been validated against MRI. It should be noted however that the control group's CSA_{PT} is similar to that presented previously using both ultrasonography (Onambélé et al., 2007; O'Brien et al., 2010b) and MRI (Carroll et al., 2008; Seynnes et al., 2009).

Despite some potential measurement error of CSA_{PT} in the group with Achondroplasia, there is a degree of confidence that the difference in patella tendon compliance would persist despite the higher measurement variance likely to be observed when using ultrasonography compared to MRI.

For the kinematic assessment of lower limbs during gait, a Plug-in-Gait model was used in both groups. The consistently flexed knee position of the group with Achondroplasia may have been brought about by the estimation of their hip joint centre (HJC), which is explained in detail in the Discussion of Chapter 7 and in this Chapter (Section 8.4.1). While Broström et al. (2009) present data to suggest the HJC predictions of individuals with Achondroplasia are different between three methods (Plug-in-Gait, functional and regression), only four individuals were included in their study. To the author's knowledge, the data of Broström et al.'s group remain the only available data that indicates a prediction of HJC in any population with Achondroplasia. In addition, only one participant from Broström's study appears to be comparable to the cohort of individuals with Achondroplasia included in this thesis. While Plug-in-Gait may incur error of HJC prediction in individuals with Achondroplasia and, in turn, miscalculate knee angle of the group during ait, there is no specific model to ascertain the joint kinematics of the group in the literature.

8.7 Implications of the thesis and future recommendations

This thesis presents a number of novel findings related to physiological and biomechanical function in an adult population with Achondroplasia. The results from

this thesis identified three main challenges for the population that are the scope of future work. Firstly, clinical descriptions of the condition appear inaccurate in their current form. For example, the relative presentation of body composition lessens some of the clinical classifications commonly given to the population when presented as total-body values. Secondly, exercise interventions may be a viable option for clinicians to pursue in order to improve some of their functional measures from this thesis. Lastly, throughout this thesis the methods employed to measure all variables were chosen based on their development and validity within control populations. There appears to be no validated methods of functional measures for the populations with Achondroplasia. The following sections of this discussion will focus on these three implications.

8.7.1 Large reference data sets are required for populations with Achondroplasia

Clinical descriptions of an individual are based on the comparison of a physical parameter to a reference data set (T. Kelly et al., 2009). For many of the anthropometric measures made in Chapter 2, the group with Achondroplasia appear to be at a heightened risk of a number of health complications compared to controls (Hecht et al., 1987; Wynn et al., 2007; Matsushita et al., 2016). When the data were presented relative to total-body and total-limb measures however, this was not the case. The commonly used normative data sets to define health states of controls populations are valid given the similarity in the populations used to provide such data (T. Kelly et al., 2009). However, comparisons are only valid when they are between populations of similar age, sex and stature, of which there are ample control data.

For accurate and valid descriptions of clinical states in adults with Achondroplasia to be made, large normative data sets of the population are required. Currently, only age for height and age for weight data of populations ≤18-years-old are available for populations with Achondroplasia (Horton et al., 1978b; Hecht et al., 1988; Hunter et al., 1996a).

8.7.2 Exercise interventions

There are ample data available in the literature that link resistance, high impact and aerobic exercise to improvements in many of the physiological and biomechanical variables measured in this thesis. As examples: BMD of the femoral neck and lumbar column increase following resistance, high impact and aerobic exercise interventions in young, menopausal and elderly women (Vincent and Braith, 2002; Vainionpää et al., 2005; Mosti et al., 2014; Beavers et al., 2017); resistance training leads to improvements in both force production and tendon stiffness and is observed in controls (Kubo et al., 2001a; Kubo et al., 2001b; Onambélé et al., 2008; Seynnes et al., 2009), the elderly (Reeves et al., 2003a; Reeves et al., 2003b; Onambélé et al., 2006; Onambélé et al., 2008) and children (Waugh et al., 2014); cardiovascular training improves $\dot{V}O_{2max}$ in controls (Nybo et al., 2010), individuals with GHD (Cuneo et al., 1991; Woodhouse et al., 1999), children (Carazo-Vargas and Moncada-Jiménez, 2015), the obese (Verheggen et al., 2016) and clinical groups (Gjellesvik et al., 2012); there is also a consensus that steady state submaximal exercise (~65% $\dot{V}O_{2max}$) induces fat metabolism (Achten and Jeukendrup, 2004) and alters mass distribution (both muscular and adiposity). Despite the wealth of exercise

interventions conducted on controls, there appears to be no equivalent data in any population with Achondroplasia.

Based on the results from Chapter 2 and the assumption of a larger relative ground reaction force (N/kg) during walking and running of an individual with Achondroplasia compared to controls, the bone turnover of individuals with Achondroplasia is similar to controls; this is based on the shank and foot BMC and BMD relative to the respective total-leg values. An assumption can be made therefore that improvement of other tissues, such as muscle and tendon, could be made through exercise in populations with Achondroplasia. Through such interventions, BMD, $\dot{V}O_{2max}$, force production and tendon compliance are likely to improve strength imbalance (such as that observed between the hip flexors (hamstrings) and extensors (quadriceps) in Chapter 5), lower the risk of falls (Onambélé and Degens, 2006; Onambélé et al., 2008), increase the likelihood of adherence to regular habitual physical activity (Weinsier et al., 2000), improve walking and running C (Albracht and Arampatzis, 2013; Arampatzis et al., 2006; Fletcher et al., 2010) and in turn improve quality of life and lower the risk of health complications (Wilmot et al., 2012).

It would be reasonable to suggest from the available data in controls, that exercise interventions within populations with Achondroplasia may improve some of their functional measures made within this thesis. However, with Achondroplasia affecting bone end-plate development and structure, it is likely that the ability of a person with the condition to perform complex resistance exercises is different to

controls. Therefore, it would be advised that movement analyses of different exercises be explored in the populations with Achondroplasia prior to intervening with previously utilised exercise modes.

8.7.3 Methodological improvements in research for populations with Achondroplasia

As discussed in Chapter 7, and earlier in the current Chapter (Section 8.6), the measurements of physiological and biomechanical variables, such as the joint centre predictions and body segment masses, may not be as accurate as would be ideal to optimise the accuracy and validity of the reported data. Conventional gait models, such as Plug-in-Gait, were developed using populations similar to the control group included in this study (Davis et al., 1991). The genetic mutation that leads to Achondroplasia predominantly affects the growth plates of the long bones. Both the proximal and distal ends of the long bones appear deformed compared to controls (Ponseti, 1970). Certainly, the data presented by Aykol et al. (2015) and those presented in Chapter 5, show that the knee morphology of individuals with Achondroplasia is different to that of controls. Broström et al. (2009) presented data to suggest the HJC predictions are different between different prediction methods (Plug-in-Gait, regression and functional) in individuals with Achondroplasia. Therefore, for more valid descriptions of gait, appropriate anatomical models of in individuals with Achondroplasia that provide accurate and valid predictions of the lower limb joint centres are required. An example of such a technique would be to use a real time dual-arm fluoroscopy device, although this would expose the participant to unnecessary amounts of radiation.

The data presented in Chapter 2 were collected using dual energy X-ray absorptiometry. While the methods used to measure body composition and the methods of segmenting the scans are reliable (Durkin et al., 2002; Durkin and Dowling, 2003; Glickman et al., 2004), other methods, such as MRI or computer tomography, are likely to quantify total-segment masses more accurately than DEXA. The 3D imaging of segments using MRI or computer tomography would also allow for inertial segment characteristics to be made. These could then be used for more accurate modelling of movements, such as gait, and biomechanical variables, such as energetics, to be more accurately calculated in individuals with Achondroplasia. Whilst the measurement and use of inertial parameters and energetics were beyond the scope of this thesis, this body of work provides a caveat that normative *in vivo* data sets or more valid methods be used to improve the accuracy of results in these areas. The development of methodology in research for individuals with Achondroplasia would help validate the measures made in this thesis and provide more robust descriptions for the population as a whole.

8.8 Conclusion

This thesis has provided novel *in vivo* anthropometric and neuromuscular data of adult males with Achondroplasia. Furthermore, this thesis has extended the available gait related kinematic data for an adult population with Achondroplasia, with what appears to be the first to provide gait kinematics in an adult population without leg lengthening surgery. Many novel observations were made in all experimental Chapters. Firstly, total-body and segmental anthropometry and body composition

differ to controls, with the group with Achondroplasia being classified as osteopenic on the current clinical guidelines; although the definition was changed when presented relative to total-body and total-limb masses. $\dot{V}O_{2max}$ was lower as an absolute measure in the group with Achondroplasia compared to controls, but when expressed relative to TBM and FFM, differences between groups were again removed. $\dot{V}O_2$ during walking and running was higher in the group with Achondroplasia and was explained by their shorter legs. The walking and running C in the group with Achondroplasia was consistently higher than controls despite accounting for body mass and leg length. The specific force of VL and patella tendon compliance during knee extension iMVC was lower in the group with Achondroplasia compared to controls. In addition, during knee extension iMVC, the antagonists (here as the bicep femoris) were more active in the group with Achondroplasia than controls. Numerous differences in gait kinematics were observed between groups, which led to the group with Achondroplasia being more 'flexed' at the pelvis, hip, knee and ankle during walking, and more flexed at the pelvis and knee during running only compared to controls. A combined gait kinematic score (GPS) showed that gait of the group with Achondroplasia was quantifiably different to controls during walking and running; although the two groups were more similar when running. The combination of a greater upper body mass distribution, lower specific force, lower tendon compliance and greater differences in gait kinematics of the group with Achondroplasia are likely to contribute to their higher C during walking and running. The work within this thesis should undoubtedly aid clinicians in the assessment of anthropometry within individuals with Achondroplasia and act as a reference for future populations when performing functional tasks.

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Appendices

Appendix 1 – Informed Consent and Information for Participant Sheet.

Department of Exercise and Sport Science

Informed Consent Form

(Both the investigator and participant should retain a copy of this form)

Name of Participant:

Supervisor/Principal Investigator: **David Sims (david.sims@stu.mmu.ac.uk)**

Project Title: **The neuromuscular and kinematic analysis of adult males with congenital skeletal dysplasia.**

Ethics Committee Approval Number: 11.03.14(i)

Participant Statement

I have read the participant information sheet for this study and understand what is involved in taking part. Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without giving a reason. Any concerns I have raised regarding this study have been answered and I understand that any further concerns that arise during the time of the study will be addressed by the investigator. I therefore agree to participate in the study.

It has been made clear to me that, should I feel that my rights are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the Registrar and Clerk to the Board of Governors, Head of Governance and Secretariat Team, Manchester Metropolitan University, All Saints Building, All Saints, Manchester, M15 6BH, Tel: 0161 247 1390 who will undertake to investigate my complaint.

Signed (Participant)		Date	
Signed (Investigator)		Date	

Parental or guardian consent for research involving children.

I confirm that the details of this study have been fully explained and described in writing to (insert name) and have been understood by him/her and I therefore consent to his/her participation in this study.

Signed :		Date :	

Please provide a contact number in case we need to get in touch with you.

Telephone :

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Information Sheet for Participants

Title of Study:

The neuromuscular and kinematic analysis of adult males with congenital skeletal dysplasia.

Ethics Committee Reference Number: 11.03.14(i)

Information Sheet

1) This is an invitation to take part in a piece of research.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?

The purposes of the study are: 1) to identify any differences between the muscle structure, function and walking patterns between dwarf and average-statured young adults and 2) to determine whether any group difference remains when muscle size and stature are accounted for.

3) Why is the study being performed?

There is limited data on the walking patterns, muscle structure and function, and fitness of individuals with skeletal dysplasia. This study aims to assess and describe these measures in individuals with skeletal dysplasia against adults of an average stature while performing relevant activities that include: maximal strength, walking and running Analysis of physiological and biomechanical variables during these tasks will help in identifying limiting factors to gait and muscle strength in individuals with SD.

4) Why am I being asked to take part?

You have been invited to partake in this research because you fit the inclusion criteria to be in one of the following groups:

1. Skeletal Dysplasia adult
2. You are less than 1.48m in stature, you are free from any neuromuscular condition or injury, you are aged 18-35
3. Average-statured adult
4. You are above 1.48m in stature, you are free from GH deficiency or any growth disorders, you are free from any neuromuscular disorder or injury, you are aged 18-35, and your habitual physical activity levels match those of our Skeletal Dysplasia group

The data from this study will inform applied scientist, physicians and physiotherapists on mobility in skeletal dysplasia. Our findings will also inform future research in this area.

5) Do I have to take part?

You are under no obligation to take part in this study. If, after reading this information sheet and asking any additional questions, you do not feel comfortable taking part in the study you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you and this will not be included when the research is reported. If you decide not to take part or withdraw from the study, this will not affect your relationship with any of the staff at the Manchester Metropolitan University, Dream It Believe It Achieve It, who is funding the programme, or Dwarf Sports Association.

If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part. You will be given a copy of the consent form together with this information sheet to keep.

6) What will happen to me if I agree to take part?

You will be asked to partake in one testing session where you will undergo the following tests (order not relevant):

VO_{2max}: This test will involve you running on a treadmill. This test will get harder every three minutes until you cannot carry on running. At this point the test will be stopped. During the test you will wear an apparatus (essentially a face mask)

attached to the front of your face (nose to chin) for you to breathe into, with the breathing tube partly resting on your chest. This will measure how much air you are breathing in and out per minute. At the end of this test you will feel tired and sometimes dizzy or sick. This feeling will pass after a few minutes. The mask that is used to collect gas can make some feel hot and/or claustrophobic.

Exercise economy: Again you will wear a portable machine that measures gas inhalation and exhalation. You will walk for 5mins and then run at a low intensity for 5mins whilst gas is collected. After the test you may feel hot and/or claustrophobic due to the mask, but the test is not strenuous.

Biomechanical analysis: This test will involve you having reflective markers (small circular silver coloured balls) stuck using double sided sticky tape on your skin and then walking and running over a force plate. This is to measure how fast each body part is moving, as well as to estimate how much force you are putting through your joints. These tests are at very low intensities and you should not feel any discomfort other than normal walking and running.

Muscle and tendon architecture and force production: This is a test that will measure your: quadriceps muscle strength (thigh muscle), volume and size; and your patella tendon length and size (knee tendon).

Firstly, your muscle and tendon size will be measured using ultrasonography. This is a non-invasive test that requires no exertion from you. You will then be strapped into a chair with your left leg attached to a machine that measures force. You will push against the machine as hard as you can (it will not move) and your left quadriceps force production will be measured. You may feel some muscle fatigue at the end of this test. During the test, you will have a small electrical current passed through the working muscle; this is called electrical stimulation. Some may feel some discomfort during this part of the procedure. The electrical charge will be passed through two pads that are stuck onto the skin, on the thigh muscle area. In addition to this other markers will also be stuck onto your skin. These are electromyography electrodes and they measure the electrical activity in your muscle; your skin will be shaved of hair where the electrodes will be placed. The electrodes are entirely passive and so there is no discomfort felt during this part of the test. At any point, you may tell the investigator(s) that you are in discomfort and that you want to stop the test.

DEXA scan- This procedure is somewhat similar to that of a medical X-ray. You will be asked to lie on a bed and remain as still as possible throughout the scan which will last approximately 10 minutes. Each DEXA scan exposes you to an extremely minimal dose of radiation, which is well below the maximum recommended dose regarded as safe (see question 7 for more details).

7) Are there any disadvantages or risks in taking part?

Should you decide to take part in this research, you will be exposed to approximately 5µSv of radiation during assessment on the DEXA scanner. This dose

is extremely minimal and is equivalent to half the amount of natural background radiation you are subjected to over an average day. The IVA scan that will be used for assessment of the knee will expose you to around 2µSv.

Throughout all tests you may feel slight discomfort. The VO_{2max} test will exert you the most and you will feel most tired and possibly nauseous afterwards. This feeling will last for 10-15mins depending on the individual. Some may feel discomfort at the electrical stimulation but this is very short lived and again, at any point, you may tell the investigator(s) to stop the test at any point.

8) What are the possible benefits of taking part?

The benefits of the study may not relate directly to you, but will help increase the knowledge our understanding of skeletal dysplasia. The study aims to increase the knowledge of this group as well as opening further areas to research in the future. You may request immediate feedback of your tests, however full analysis of all participants will need to be carried out to give reliable feedback regarding the group you are in.

9) Who are the members of the research team?

The Principal Investigator of the study is David Sims (david.sims@stu.mmu.ac.uk). There are three supervisors in the team and these include:

Dr. Christopher Morse
Dr. Adrian Burden
Dr. Gladys Pearson

10) Who is funding the research?

The funding for these studies comes from the '*Dream It Believe It Achieve It*' charity that works closely with UK Dwarf Sports Association. The principal investigator is an employee of '*Dream It Believe It Achieve It'.*

11) Who will have access to the data?

All information collected during the course of the research will be kept confidential and will only be used for the purposes of the study. All data will be stored on a password-protected computer and the MMU secure network with your identity kept anonymous.

The results of the study are likely to be communicated at conferences or published in scientific journals at some point in the future but in a manner that does not allow an individual's identity to be determined. You may at any point ask to have your data removed from the study and/or publications. You are also entitled to a copy of any publication with your data included. To ask for copies please contact:

David Sims (07169858@stu.mmu.ac.uk)

12) Who do I contact if I feel my rights have been violated?

MMU Ethics Committee
Registrar & Clerk to the Board of Governors
Head of Governance and Secretariat Team
Manchester Metropolitan University
All Saints Building, All Saints
Manchester M15 6BH
Tel: 0161 247 1390

I confirm that the insurance policies in place at Manchester Metropolitan University will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

13) Finally, a thank you!

Thank you for considering partaking in this study.

Appendix 2 – Plug-in-Gait marker set up.

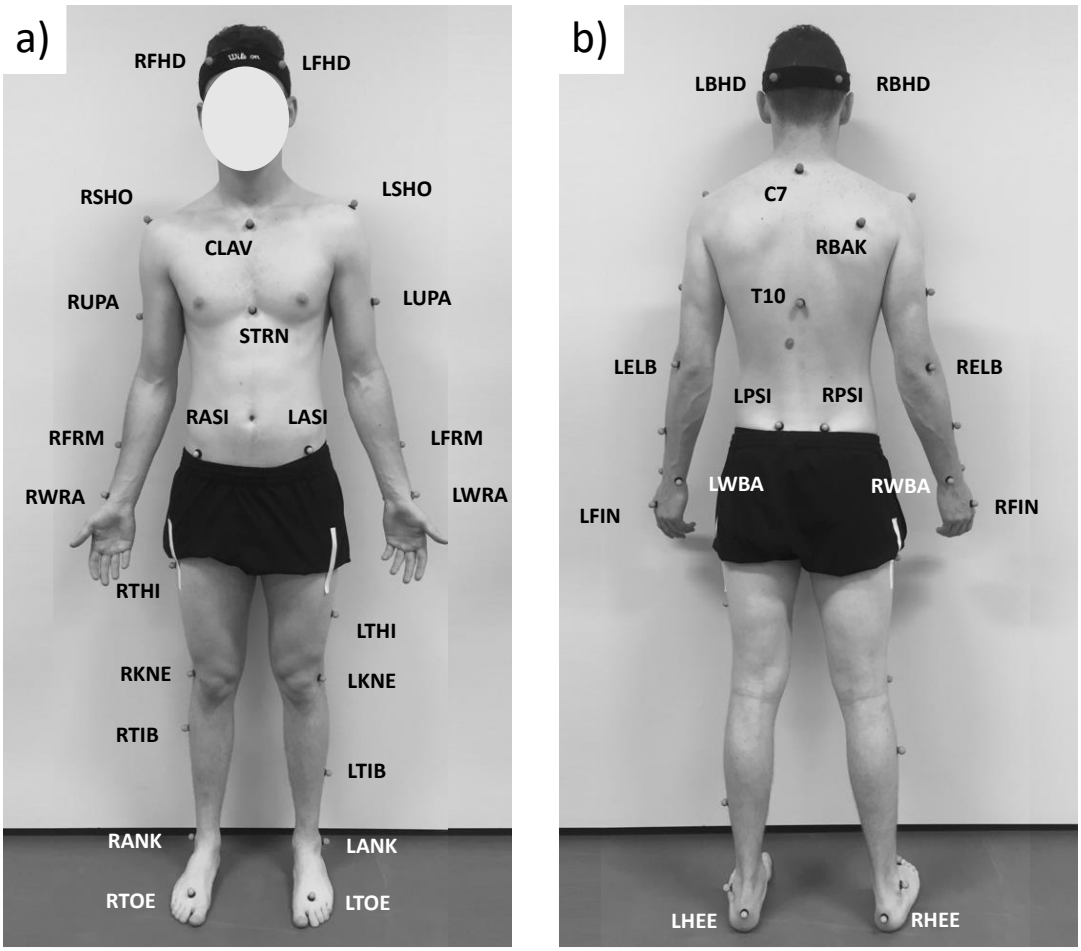


Figure A2.1: An example of the Plug in Gait Marker set for one control. Descriptions of the placement for each marker are given in Table A2.1. Note: this figure includes an 'L' and an 'R' in front of the marker acronym to define the left and right side, respectively. In some cases, tape (Zinc Oxide) was used to keep clothing from obstructing markers.

Table A2.1: Marker definitions, locations and identifications for the Plug-in-Gait marker set.

Marker Acronym	Defines the:	Location	Identification
FHD	Origin and plane of the head	Front of the head	Located approximately over the left temple.
BHD	Together with the FHD, define the orientation of the head	Back of the head	Placed on the back of the head, in a horizontal plane of the front.
C7	C7, CLAV, T10 and STRN define the sagittal plane of the torso	Spinous process of the 7th cervical vertebrae	The most prominent spinal process on the back of the neck.
CLAV		Jugular Notch where the clavicle meets the sternum	The marker should be placed on the bone and not in the jugular notch.
T10		Spinous Process of the 10th thoracic vertebrae	This marker position is located by finding the inferior angle of the scapula. Move horizontally across to the vertebrae. This should be T7. Palpate three vertebrae inferiorly.
STRN		Xiphoid process of the Sternum.	Placed on the bone just above the Xiphoid process.
RBAK	Rotation of the torso	Scapula	Mid scapula
SHO	Glenohumeral joint	Acromio-clavicular joint	
UPA	Plane of the humerus (upper arm)	Over the upper (right) and lower (left) lateral 1/3 surface of the upper arm.	In line with the projection from SHO and ELB markers.
ELB	Elbow joint	lateral epicondyle approximating elbow joint axis	
FRA	Plane of the fore arm	Over the upper (right) and lower (left) lateral 1/3 surface of the fore arm.	In line with the projection from ELB and FIN markers.
WRA	Wrist joint	Styloid process of the radius (thumb side)	
WRB		Styloid process of the ulna (little finger side)	
FIN	Axes of the hand	2nd Metatarsal head	Placed on the dorsum of the hand just below the head of the 2nd metatarsal

ASI	HJC and pelvic axes.	Anterior Superior Iliac Spine.	Directly over the anterior superior iliac spines.
PSI	Together with ASI, define the axes of the pelvis.	Posterior Superior Iliac Spine.	Bony prominences which are below the sacro-iliac joint (dimple).
THI	Plane of the femur (thigh)	Over the upper (right) and lower (left) lateral 1/3 surface of the thigh.	In line with the projection from HJC (greater trochanter) and KNE marker.
KNE	Knee Joint	Lateral epicondyle	
TIB	Plane of the tibia (shank)	Over the upper (right) and lower (left) lateral 1/3 surface of the shank.	In line with the projection from KNE and ANK markers.
ANK	Ankle Joint	Lateral malleolus	Most lateral aspect of the bony process.
TOE	Toe (anterior of foot and dorsal surface) - Together with the HEE, defines the plane of the foot.	Dorsal surface of the foot.	Head of the second metatarsal head.
HEE	Heel (posterior of foot) - Together with the TOE, defines the plane of the foot.	Most posterior part of, and central of, the calcaneus.	Height is not important but must be aligned with the TOE marker to define the long axis of the foot.

To distinguish between markers and their definitions, the central markers are shown in white, the upper appendicular markers are shown in light grey and the lower appendicular markers are shown in dark grey.

Appendix 3 – Worked example of the Gait Profile Score

Table A3.1: An example of the knee flexion/extension gait variable score at self-selected walking. Note: this example is for a single gait cycle splined to 20 data points.

A ($x_{i,t}$)	C ($\bar{x}_{i,t}^{ref}$)	Gait Variable Score Calculation			
Knee Flexion (°)	Knee Flexion (°)	$A - C^2$	$\sum A - C^2$	$\frac{\sum A - C^2}{20}$	$\sqrt{\frac{\sum A - C^2}{20}}$
11.0	5.8	26.9	3704.0	37.0	6.1
20.9	12.6	67.9			
28.3	16.5	137.8			
27.6	16.1	132.5			
21.0	12.6	71.3			
13.9	8.3	31.8			
9.7	4.5	27.1			
9.2	2.0	51.3			
11.4	1.5	98.6			
16.2	4.1	144.1			
25.6	11.2	207.9			
41.2	22.6	346.6			
59.3	37.1	494.1			
71.9	49.8	486.8			
72.9	53.5	377.5			
64.1	46.8	299.8			
50.5	32.9	310.7			
33.5	17.2	263.1			
15.4	4.9	110.6			
5.0	0.9	16.3			

A, participant with Achondroplasia (ith); C, control ($\bar{x}$).

Table A3.2: An example of the gait profile score calculation of a single participant with Achondroplasia at self-selected walking speed.

GVS	GVS (°)	GVS^2	$\sum GVS^2$	$\frac{\sum GVS^2}{15}$	$\sqrt{\frac{\sum GVS^2}{15}}$
Left Pelvis Sagittal	4.9	23.9	2248.3	149.9	12.2
Left Hip Sagittal	3.5	12.0			
Left Knee Sagittal	13.5	182.8			
Left Ankle Sagittal	9.4	88.7			
Left Pelvis Frontal	2.4	5.9			
Left Hip Frontal	3.6	13.1			
Left Pelvis Transverse	9.4	88.3			
Left Hip Transverse	29.0	838.4			
Left Foot Transverse	1.7	3.0			
Right Hip Sagittal	7.9	62.8			
Right Knee Sagittal	16.4	268.9			
Right Ankle Sagittal	10.0	100.7			
Right Hip Frontal	5.4	29.4			
Right Hip Transverse	23.0	527.3			
Right Foot Transverse	1.8	3.1			

Appendix 4 – Inferential statistics associated with Chapter 7

Table A4.1: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the pelvis (P1-7) and average pelvis position at each walking speed.

Variable	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
P1	0.016	0.038	0.036	0.027	0.016	0.015	0.005	0.020
P2	0.009	0.019	0.016	0.027	0.014	0.003	0.002	0.010
P3	0.007	0.015	0.013	0.018	0.009	0.003	0.004	0.007
P4	0.490	< No main effect >						
P5	0.430	< No main effect >						
P6	0.015	0.436	0.602	0.023	< 0.001	< 0.001	0.001	0.061
P7	0.003	0.795	0.121	0.009	< 0.001	0.002	0.002	0.005
Average Joint Position	0.006	0.01	0.014	0.017	0.006	0.004	0.004	0.007

ME, Main Effect

Table A4.2: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the hip (H1-9) and average hip position at each walking speed.

Variable	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
H1	0.295	< No main effect >						
H2	0.098	< No main effect >						
H3	0.232	< No main effect >						
H4	0.260	< No main effect >						
H5	0.344	< No main effect >						
H6	0.771	< No main effect >						
H7	0.739	< No main effect >						
H8	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.001	< 0.001
H9	0.100	< No main effect >						
Average Joint Position	0.037	0.087	0.066	0.126	0.018	0.006	0.027	0.063

ME, Main Effect

Table A4.3: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the knee (K1-7) and average knee position at each walking speed.

Variable	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
K1	0.892	< No main effect >						
K2	0.008	0.044	0.001	0.004	0.023	0.092	0.023	0.448
K3	0.005	0.040	< 0.001	0.004	0.023	0.078	0.019	0.378
K4	0.000	< 0.001	< 0.001	0.001	0.001	0.002	0.001	< 0.001
K5	0.704	< No main effect >						
K6	0.036	0.073	0.118	0.080	0.034	0.023	0.024	0.034
K7	0.005	0.035	0.007	0.013	0.006	0.002	0.002	0.007
Average Joint Position	0.004	0.009	0.01	0.01	0.002	0.004	0.003	0.012

ME, Main Effect

Table A4.4: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the ankle (A1-9) and average ankle position at each walking speed.

Variable	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
A1	< 0.001	0.002	0.002	< 0.001	< 0.001	< 0.001	0.001	< 0.001
A2	< 0.001	< 0.001	0.001	0.001	0.001	0.002	< 0.001	< 0.001
A3	0.112	< No main effect >						
A4	0.594	< No main effect >						
A5	0.636	< No main effect >						
A6	0.036	0.073	0.118	0.080	0.034	0.023	0.024	0.175
A7	0.602	< No main effect >						
A8	0.916	< No main effect >						
A9	0.473	< No main effect >						
Average Joint Position	< 0.001	< 0.001	0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001

ME, Main Effect

Table A4.5: Main effects and pairwise comparison P values for the between group comparisons of centre of mass displacement in the medio-lateral direction as a total displacement and in the vertical direction from the initial heel contact at each walking speed.

Variable	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
Medio-lateral	0.008	0.058	0.234	0.050	0.003	0.231	0.019	0.007
Vertical to max height of left stance	0.001	0.262	0.017	< 0.001	0.020	0.007	< 0.001	< 0.001
Vertical to minimum height of double support	0.357	< No main effect >						
Vertical to max height of right stance	0.003	0.491	0.561	0.027	0.044	0.007	< 0.001	0.006

ME, Main Effect

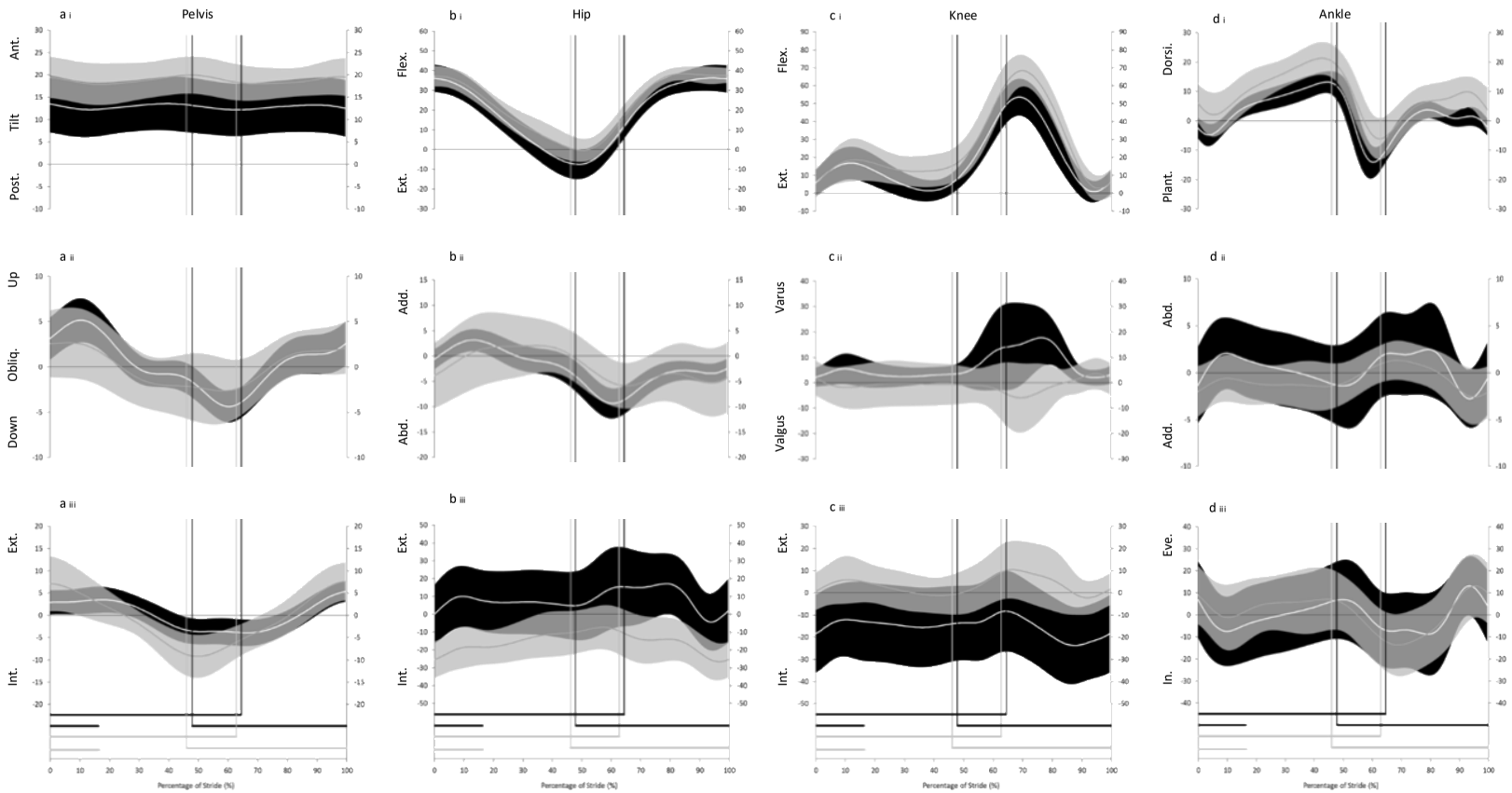
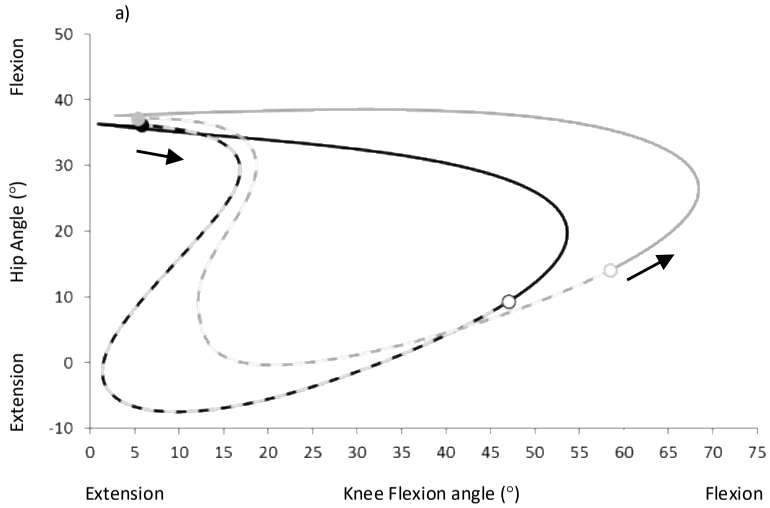
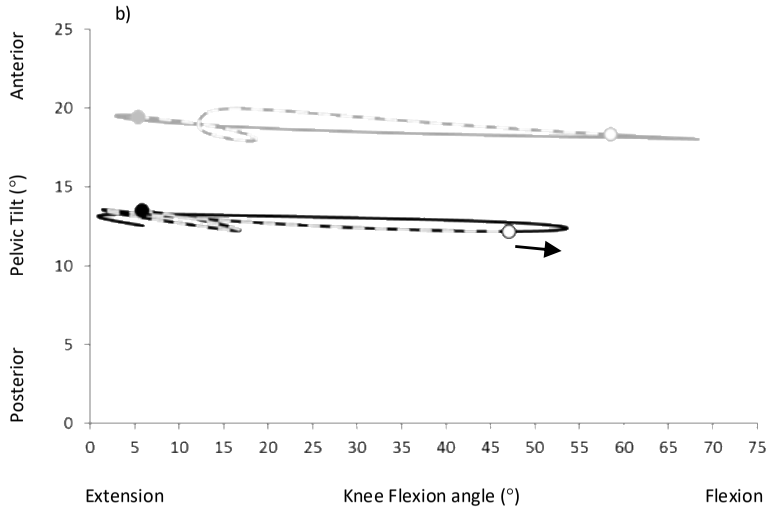


Figure A4.1: Kinematic variations of a) pelvis, b) hip, c) knee, and d) ankle over the same complete stride (%) for the i) sagittal, ii) frontal, and iii) transverse planes. Grey solid line with grey shading represents the mean (SD) of the group with Achondroplasia, while white solid line with black shading represents the control group's mean (SD), respectively. Temporal events are displayed at the bottom of each trio of graphs displaying the: top line) left contact time and bottom line) right contact time. Grey is Achondroplasia and black control. Vertical lines represent the respective heel contact and toe off points for each leg and are provided for visual interpretation.





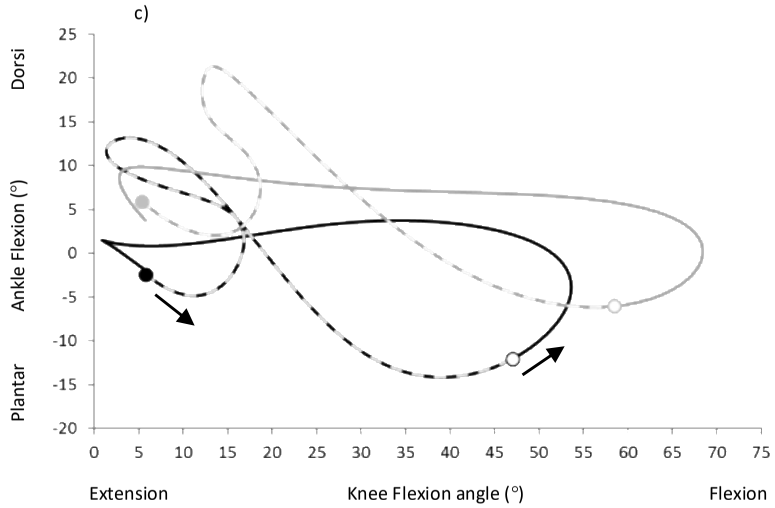


Figure A4.2: Angle-angle plots showing the kinematic pattern of a) knee flexion and pelvic tilt, b) knee flexion and hip flexion/extension, and, c) knee flexion and ankle planta/dorsiflexion during an entire stride (%). Grey and black lines represents the respective mean of the group with Achondroplasia and controls. Solid dot is initial heel contact, open dot is toe off and dashed line is the period of stance time, respectively. Arrows represent the direction of angular change.

Table A4.6: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the pelvis (P1-7) and average pelvis position at each running speed.

	ME	1.67	1.96	2.22	2.50	2.78	3.06	3.33
P1	0.084	< No main effect >						
P2	0.082	< No main effect >						
P3	0.057	< No main effect >						
P4	0.123	< No main effect >						
P5	0.657	< No main effect >						
P6	0.166	< No main effect >						
P7	0.013	0.288	0.005	0.003	0.160	0.042	0.281	0.006
Average Difference in Joint Position	0.038	0.034	< 0.001	0.104	0.041	0.161	0.240	0.331

ME, Main Effect

Table A4.7: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the hip (H1-9) and average hip position at each running speed.

	ME	1.67	1.96	2.22	2.50	2.78	3.06	3.33
H1	0.641	< No main effect >						
H2	0.689	< No main effect >						
H3	0.789	< No main effect >						
H4	0.258	< No main effect >						
H5	0.412	< No main effect >						
H6	0.261	< No main effect >						
H7	0.299	< No main effect >						
H8	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001
H9	0.008	0.020	0.007	0.005	0.012	0.006	0.017	0.007
Average Difference in Joint Position	0.570	< No main effect >						

ME, Main Effect

Table A4.8: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the knee (K1-7) and average knee position at each running speed.

	ME	1.67	1.96	2.22	2.50	2.78	3.06	3.33
K1	0.532	< No main effect >						
K2	0.494	< No main effect >						
K3	0.008	0.090	0.077	0.003	0.029	0.045	0.007	0.006
K4	0.155	< No main effect >						
K5	< 0.001	0.002	0.002	< 0.001	0.001	< 0.001	< 0.001	< 0.001
K6	0.012	0.052	0.041	0.009	0.014	0.007	0.008	0.011
K7	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001
Average Difference in Joint Position	0.028	0.141	< 0.001	0.066	0.331	0.088	0.051	0.347

ME, Main Effect

Table A4.9: Main effects and pairwise comparison P values for the between group comparisons of discrete kinematic measures of the ankle (A1-9) and average ankle position at each running speed.

	ME	1.67	1.96	2.22	2.50	2.78	3.06	3.33
A1	0.170	< No main effect >						
A2	0.559	< No main effect >						
A3	0.519	< No main effect >						
A4	0.057	< No main effect >						
A5	< 0.001	0.002	0.002	< 0.001	0.001	< 0.001	< 0.001	< 0.001
A6	0.008	0.025	0.015	0.010	0.005	0.008	0.005	0.006
A7	0.203	< No main effect >						
A8	0.544	< No main effect >						
A9	0.852	< No main effect >						
Average Difference in Joint Position	0.006	0.015	0.001	0.009	0.011	0.026	0.163	0.052

ME, Main Effect

Table A4.10: Main effects and pairwise comparison P values for the between group comparisons of centre of mass displacement in the medio-lateral direction as a total displacement and in the vertical direction from the initial heel contact at each running speed.

Variable	ME	0.56	0.83	1.11	1.39	1.67	1.96	SSW
Medio-lateral	0.551	< No main effect >						
Vertical to minimum height of left stance	< 0.001	< 0.001	0.002	< 0.001	0.003	0.007	< 0.001	< 0.001
Vertical to max height during first flight phase	0.002	0.002	0.360	0.115	0.069	0.001	0.170	0.010
Vertical to minimum height of right stance	0.006	0.062	0.033	0.009	< 0.001	0.676	0.002	0.124
Vertical to max height during second flight phase	< 0.001	0.003	0.003	0.003	0.033	< 0.001	0.010	0.001

ME, Main Effect